LONGITUDINAL ANALYSES OF MEDICATION ADHERENCE DATA IN HIV-INFECTED ILLICIT DRUG USERS

by

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Abstract

It has been long established that adherence to highly active antiretroviral therapy (HAART) is extremely important for therapeutic outcomes for the treatment of HIV-infection. With the advent of HAART, decreases in morbidity and mortality have been observed in HIV-infected individuals. However, these decreases have not generally been extended to injection drug users (IDUs) whom have additional barrier to access HAART, including adherence. Strategies such as directly observed therapy (DOT) have been successful in treating IDUs, especially when they are used in conjunction with methadone maintenance programs. Within these programs HAART and methadone are co-administered, which makes it possible to estimate HAART adherence through a surrogate, methadone adherence. With this in mind we sought to assess if ongoing illicit drug use within such a program would affect methadone adherence. Decreased methadone adherence was noted during periods of opiate use. Furthermore, we sought to assess the relationships between HAART adherence and each of virologic suppression and the emergence of drug resistance mutations. In the former, robust rates of virologic suppression were observed at every adherence level during maintenance therapy with the exception of the 80-90% adherence level where higher rates of virologic failure were observed. This may be explained by a phenomena observed when assessing the latter where higher rates of the accumulation of new drug resistance mutations were observed at the 80-90% level. Although this intermediate level of adherence has been previously described, the estimated range of adherence values reported in this thesis is much narrower than those reported in the literature.

All such analyses were conducted in a longitudinal framework, with data analysis done using generalized estimating equations (GEE). Although a cross-sectional approach is typically conducted for these types of studies, a longitudinal approach is better able to localize the outcomes (e.g. virologic suppression) to the periods and patterns of non-adherence. This may explain some of the differences found in the studies contained in this thesis which may disagree with the findings in previous reports. With this in mind, it may be important to look at adherence to HAART in a more insightful manner than has been traditionally done.

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List of Abbreviations

Abbreviation	Description
HIV	Human Immunodeficiency Virus
AIDS	Acquired Immunodeficiency Syndrome
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
ARV	Antiretroviral
PI	Protease Inhibitor
NRTI	Nucleoside Reverse Transcriptase Inhibitor
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
HAART	Highly Active Antiretroviral Therapy
PCR	Polymerase Chain Reaction
DRM	Drug Resistance Mutation
IDUs	Injection Drug Users
HCV	Hepatitis C Virus
DOT	Directly Observed Therapy
TB	Tuberculosis
CFE	BC Center for Excellence in HIV/AIDS
DTP	Drug Treatment Program
MSP	Medical Services Plan
GEE	Generalized Estimating Equations
GC/MS	Gas Chromatography/Mass Spectrometry
SD	Standard Deviation
IQR	Interquartile Range
CI	Confidence Interval
OR	Odds Ratio
RR	Relative Rate

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1 Introduction

1.1 Human Immunodeficiency Virus (HIV) in a Clinical Setting

HIV is a retrovirus known to cause the life threatening illness known as the Acquired Immunodeficiency Syndrome (AIDS). HIV is transmitted through blood-borne and sexual contact as well from mother to child during pregnancy, birth or breastfeeding. As of 2006, it is believed that 38.6 million people globally are living with the virus [1]. Although therapies exist which combat the disease, there currently is no cure.

After the virus is transmitted to a new host, it infects cells involved in the host's immune system. Once the virus enters the cell, the virus replicates its own genome using a packaged enzyme called reverse transcriptase that copies the viral RNA into cDNA. The resultant DNA is then incorporated into the cell's own genome as a provirus, where it can evade detection from a host immune response. Cell activation induces transcription of the provirus resulting in viral RNA present in the host cell's cytoplasm. The individual components required for viral maturation are subsequently transcribed and translated into viral proteins and the virus is assembled. The virus then completes its life cycle by exiting the cell along with several identical replicates of itself, a process which often destroys the cell [2].

HIV preferentially infects cells found in lymphoid tissues. A specific type of lymphocyte known as a CD4+ lymphocyte is one such cell type. In particular, CD4+ T cells or helper T cells are responsible for inducing specific immunologic response for any incoming pathogen. As the

HIV life cycle progresses sustained decreases in CD4+ cells are observed, resulting in poor immune function. Once the levels of CD4+ cells are depleted to very low levels, opportunistic infections result which usually define an AIDS diagnosis. These opportunistic microbes are almost never seen in humans with normal or even somewhat compromised immune function.

1.2 HIV Therapeutics

Soon after the isolation of the HIV virus, agents were developed to disrupt its life-cycle. These agents, known as antiretroviral (ARV) medications, can be classified into at least four different classes. Drugs have been developed to inhibit its entry into the cell, inhibit the transcription of its own genome by reverse transcriptase, to inhibit its integration into the host cell's genome, and to inhibit its maturation by an enzyme called protease. Of these four classes, protease inhibitors (PIs) and reverse transcriptase inhibitors are most commonly used in HIV therapy in 2006. The reverse transcriptase inhibitors can be subsequently classified according to their mechanism of action. Nucleoside reverse transcriptase inhibitors (NRTIs) are nucleoside analogues which are chemically similar to the nucleotides found in all organisms but lack the chemical groups necessary to bond to subsequent elements of a DNA chain. During transcription of the viral RNA to DNA, nucleosides are added in place of the regular nucleotides, effectively terminating replication. Alternatively, non-nucleoside reverse transcriptase inhibitors (NNRTIs) directly inhibit transcription by reverse transcriptase.

Despite being very potent *in vitro*, initial drugs were only successful over short periods of time after which drug resistant isolates would be selected and any therapeutic benefit of the drug would be lost. However, since the mid-1990s, significant decreases in morbidity and mortality

have been observed, which has been attributed to the development of highly active antiretroviral therapy (HAART) [3]. HAART consists of three or more ARV drugs in combination, usually with two NRTIs, and either one PI or one NNRTI. With the advent of HAART, HIV which was once considered a terminal illness, in most cases can be managed as a chronic disease.

1.3 HIV Diagnostic Testing and Monitoring

After HIV infection has been detected, the prevention or delay of AIDS diagnosis and death are the main clinical outcomes of interest. But, like any chronic disease, it must be monitored on an ongoing basis to determine the disease course and any possible interventions that may be required. Typically, HIV infection is managed by monitoring two surrogates: CD4+ cell count and HIV plasma viral load. CD4+ cell count is measured by flow cytometry and represents the average number of CD4+ cells present in a cubic millimetre (mm^3) of blood. HIV plasma viral load is a measure of the number of copies of HIV RNA present in a millilitre (mL) of blood after amplification by the polymerase chain reaction (PCR).

Within clinical studies, usually both outcomes are reported, as neither CD4+ cell count nor HIV plasma viral load by themselves have proven to be valid surrogate outcomes for the antiretroviral treatment of HIV-infection. However, suppression of HIV plasma viral load below the assay's detectable limit (currently 50 copies/mL) is usually the primary outcome in drug registration trials, with change in CD4+ cell count typically considered as a secondary outcome. In a clinical setting, maximal suppression of HIV plasma viral load is usually the ultimate goal of therapy, but many clinicians realize that this may not be possible in patients who are heavily drug experienced and cannot obtain viral suppression even in the most ideal circumstances. In cases

such as these, therapeutic goals are usually met if HIV plasma viral load can be significantly reduced and CD4+ cell counts increased.

Although clinical goals are usually defined in terms of CD4+ cell counts and HIV plasma goals, ongoing monitoring of changes in the virus' genome are usually undertaken in patients when clinical goals are not being met. This is usually done by genotypic and phenotypic monitoring of HIV drug resistance. A genotypic HIV drug resistance test involves sequencing the parts of the HIV genome targeted by therapeutics (typically the protease and reverse transcriptase genes). Once these sections of the genome have been sequenced they can be compared to the wild-type virus with any differences noted. Changes in the genome (mutations) may be indicative of drug resistance, however many mutations are relatively harmless polymorphisms, and may occur even without the selective pressure initiated by antiretroviral drugs. Once drug resistance mutations (DRMs) occur they can lead to increases in viral loads. However, these DRMs are usually specific to only a subset of the drugs taken in the HAART regimen and isolates containing these DRMs would still be susceptible to the other drugs contained within the regimen.

Phenotypic resistance is usually measured using an assay which measures the rate of viral replication in the presence of certain concentrations of drugs. These rates at set concentrations are then compared to the rates of replication observed in wild-type virus. Alternatively, instead of running an assay for each sample, phenotypic data is often estimated using the genotypic resistance result which is put through a proprietary algorithm or queried against a database with a large number of previously tested isolates.

Despite clear evidence of the effectiveness of HAART in improving morbidity and mortality in HIV-infected patients, initiation is usually delayed until certain clinical thresholds have been passed. Current clinical guidelines for initiation of HAART are primarily dependent on a CD4+ cell count threshold, after which the initiation of therapy is recommended. However, once HAART is initiated, it is generally believed that constant therapy will be required for the remainder of the patient's life.

1.4 Adherence to HAART

Soon after the development of HAART it became very clear that high rates of adherence were required for successful therapeutic outcomes. Adherence is usually represented as a proportion of pills taken out of those prescribed. However, this definition is usually constrained to rates of adherence less than or equal to 100%, as it would be possible to take more pills than originally prescribed. Some have advocated a stricter definition of adherence incorporating important elements of regimen compliance including dose-timing, dose quantity and regimen dietary requirements (e.g. certain drugs should be taken with a high-fat meal) [4]. These issues are usually ignored in studies which evaluate the impact of adherence on different clinical outcomes, where several methodologies have been developed to measure adherence.

Usually considered the most rigorous method of measuring adherence, electronic medication monitoring is accomplished by an electronic device in a pill bottle that senses when the pill bottle is opened, at which time it records the time and date of the event. It should be noted, however, that opening a pill bottle is not direct evidence of ingestion of pills, nor can it capture the common practice of taking several pills out for the rest of the day's or week's doses.

Furthermore, the technology is expensive. Hence electronic monitoring is usually only done on one component of the HAART regimen.

Another approach is based on pill counts. At study visits, the patients are asked to return the pill bottles with any remaining medication, the pills are counted, and adherence is determined for the time since the previous study visit. It is argued that pill counts often overestimate rates of adherence since patients may dump pills prior to the study visit. With this in mind, some have advocated using unannounced pill counts as a more objective measure of adherence. However, either method is a summary of the rate of adherence over the time period and does not supply any information about how periods of non-adherence were distributed throughout this period.

Self-reported adherence is often used in clinical studies and practice to assess patient adherence. Several questionnaires have been developed to assess adherence in a cost-effective and easy to implement manner. However, as with pill counts, it is usually thought that self-reported adherence overestimates adherence and may not be as objective as other methods of measuring adherence.

Pharmacy refill records are also often used to estimate adherence. This method uses periodic refill records of medication from pharmacies to estimate adherence. Refills of prescriptions are usually done on a monthly or quarterly timeline, meaning that if a patient picks up all their prescriptions they are considered to be 100% adherent regardless of the number of pills ingested. Therefore, this method of measuring adherence is very crude, as it only measures the ability to pick up one or more months' worth of pills and reveals nothing about how those pills are used once they have been distributed. Furthermore, claims of validity regarding this

method of measurement are region-dependent. A claim of validity made in one region does not necessarily generalize to another region which may have a completely different method of distributing pills. Each difference in the methods used to distribute medication may have a consequence on how measurements done using this method apply to patients actually taking the pills.

In the landmark study by Paterson and colleagues in 2000, it was reported that patients taking PI-based HAART with adherence below 95% had substantially higher rates of detectable viral loads [5]. This study used electronic medication monitoring and followed patients for a mean follow-up time of 6 months. After the publication of this study, the results were verified using other methods of measuring adherence [6-8]. The importance of adherence for other clinical outcomes has also been reported for genotypic resistance [6, 9-11], CD4+ cell count changes [12, 13] , progression to AIDS [14], and death [15]. Most studies indicated that the 95% adherence threshold was critical in producing optimal clinical outcomes. However, these studies were generally limited to HAART regimens not widely utilized today.

1.5 HIV and HAART in Injection Drug Users (IDUs)

Within North America, injection drug users were among the first social groups to contract HIV in large numbers. The sharing of needles is an extremely effective method of transmitting blood-borne illnesses including HIV and hepatitis C virus (HCV) and, accordingly, high rates of HIV infection are observed within injection drug using populations throughout the world. Within Canada, IDUs represent between 20-30% of new cases of HIV infection [16]. In particular, the Downtown Eastside community in Vancouver, British Columbia has experienced an epidemic of

HIV-infection only exceeded by the prevalence of HIV observed in certain communities within sub-Saharan Africa.

Unfortunately, IDUs also represent a segment of the population that are prone to poverty, mental illness and social instability. It is not surprising then that early treatment of IDUs was subject to poor adherence to HAART and generally poor clinical outcomes. With these facts in mind, physicians and therapeutic guidelines have traditionally been very cautious about initiating HAART in patients still engaging in illicit drug use. As IDUs represent a large proportion of the HIV-infected population, the development of treatment programs that can successfully cater to the needs of IDUs was, and still is, of utmost importance.

1.6 HAART Directly Observed Therapy (DOT) for IDUs

It was hypothesized that previous success with directly observed therapy (DOT) programs in other settings such as tuberculosis (TB), might extend to the treatment of HIV-infection in difficult to engage populations such as IDUs. In the TB setting, DOT was utilized as a mechanism for ensuring adherence to antibiotics while combating drug resistant TB in patients thought unable to be adherent to therapy. Within such programs, patients present themselves daily to a pharmacist or other health care professional who dispenses the medication and observes the patient ingest it. Non-attendance can be monitored and support staff can be deployed to remind patients about their medication. Within the TB setting, daily attendance is often enforced legally.

The situation is very similar for IDUs who, in the absence of support programs such as DOT, may not be able to comply with the strict HAART adherence requirements. Hence, DOT programs for the treatment of HIV-infection have been initiated by centres in Vancouver [17, 18], Baltimore [19], Los Angeles [20], and New Haven [21]. Unlike TB treatment, such programs are usually not legally enforced and, although programs have been initiated without a reinforcement mechanism, the most successful programs to date have resided within methadone maintenance programs for patients receiving therapy for opiate dependence. In these methadone maintenance programs, patients are usually already required to present themselves daily to have their methadone prescription dispensed and ingestion observed. Therefore, it's very feasible from an economic and logistical perspective to co-administer HAART with methadone during these daily visits. In particular, in regions like British Columbia where many pharmacies are approved to distribute methadone, a DOT program need not reside in a specific clinic, and patients can attend clinics or pharmacies close to their place of residence.

1.7 Methadone

Methadone, a μ-opioid agonist, is the current standard of care for the treatment of opiate dependence in Canada. Methadone is usually orally ingested; it is slowly absorbed, and remains active in the body for approximately 24 hours. It effectively blocks the opioid receptor so that if opiates are taken while methadone concentrations are still high enough no effect of the opiates is realized. This property accompanied by its long half-life makes methadone an effective substitution therapy for the treatment of opiate dependence. Psychological and physical addiction can be combated over time with appropriate counselling and reductions in methadone dose.

Furthermore, the slow absorption reduces any positive reinforcement associated with fast absorption realized with other modes of administration such as injection or smoking opiates.

Although widely available in British Columbia, methadone remains a highly controlled substance and administration records are heavily monitored and accurate. Within DOT programs, methadone and HAART are co-administered. Hence, adherence to HAART can be both monitored and measured through the methadone administration records. In patients who receive HAART regimens consisting of pills which are all dosed once a day, methadone adherence can be viewed as equivalent to HAART adherence. In recent years, there has been significant expansion in the number of ARVs which can be dosed once daily. Two independent HAART regimens can be composed of approved once-daily ARVs such that complete resistance to the first regimen does not imply failure to the second regimen.

Given patients are required to present themselves daily to a pharmacy to receive HAART and methadone, it could be possible to measure adherence to HAART directly. However, the program is structured to allow patients to receive methadone at a pharmacy of their choosing, and it is usually not possible to require pharmacist to maintain records of daily administration of HAART. Methadone, on the other hand, is heavily regulated and pharmacists are required to maintain detailed records when dispensed. The structure of the program, along with the availability of potent once-daily HAART regimens make methadone adherence a very practical alternative to measuring HAART directly.

The availability of such adherence data provides a unique opportunity to monitor adherence to HAART over a long period. Methadone adherence also has several features which

make it preferable to other methods of measuring adherence. Firstly, daily adherence is measured, which allows for the evaluation of the impact of specific patterns of non-adherence. Electronic monitoring is the only other method of measuring adherence described in the literature that also has this ability. Secondly, this method measures the proportion of pills taken out of what was prescribed. Both electronic monitoring and pill counts accomplish this as well. Unlike electronic monitoring, however, methadone adherence should extend to all elements of a HAART regimen, whereas electronic monitoring is usually only performed on the PI or the NNRTI component of the regimen. Administration records alone are not able to ensure dose timing (i.e. that a once-daily drug is taken 24 hours apart), but the half-life of methadone usually keeps patients on a regular schedule.

1.8 Overview: A Unique Opportunity

To date, most studies examining the consequences of non-adherence to HAART have been relatively short-term (usually less than one year, and often less than six months), or have used methods of measuring adherence which cannot access some of the features available using methadone adherence. A DOT program which resides in a methadone maintenance program provides an excellent opportunity to study adherence to HAART as it does not require any additional infrastructure beyond the program itself. Further, in many populations that could be studied over the long term, rates of adherence are very high and no significant variability in adherence is observed. In these study populations adherence may only be studied at the extreme high end of adherence. IDUs, on the other hand, have considerably more variability in their methadone adherence due to relapses in their addictions. The relapsing behaviour usually exhibited in IDUs lends itself nicely to a longitudinal approach, where these periods of relapse

and stability can be localized to a specific clinical observation (e.g. a HIV plasma viral load) which is proximate to that time.

With this in mind, we sought to examine several issues relating to adherence in a DOT setting. First, we sought to estimate the impact of illicit drug use (and other measured covariates) on methadone adherence. These data provide important information about patients who may be suitable for HIV treatment within a DOT program based upon their known drug use. It may also reveal which patients may require additional engagement and counselling while enrolled in a DOT program. Secondly, although there are many studies examining the relationship between adherence and viral suppression, little data have been presented in patients receiving contemporary drug regimens consisting of HAART regimens containing NNRTIs or ritonavir boosted PIs. We sought to re-examine this relationship using methadone adherence as a surrogate for HAART adherence. Thirdly, the relationship between non-adherence and how patients develop drug resistance is poorly understood. We sought to assess this relationship and to determine levels of adherence which may be particularly prone to the development of drug resistance.

2 Data Collection

2.1 Overview

Each of the analyses presented in the remainder of this thesis involve the construction of longitudinal datasets where each patient has one or more observation. In each such analysis, methadone adherence is measured and used either as a response variable or as a predictor for HIV-treatment related clinical outcomes. Each analysis was restricted to patients enrolled in the DOT and methadone maintenance programs at the Pender Community Health Center in the downtown eastside area of Vancouver. Data was collected on patients from October 2002 through May 2006. Each study has a slightly different timeline dictated mainly by when the data was last collected. In the following analyses, data was obtained by collating multiple sources of clinic data, where some data sources were only available for a short period of time.

2.2 Methadone Dose and Adherence Data

Daily methadone dose data were available through pharmacy records. Information including patient, date, dose, whether the prescription had been filled, prescribing physician, and physician instructions was entered into the research database. Data collection of the pharmacy records began in November 2003 and occurs approximately every six months. Hence, in some patients, methadone data is available as early as October 2002, with the last date of collection occurring in May 2006. Unfortunately, a comprehensive list of eligible DOT patients was not available until 2004 and for many patients eligible for the study much of their retrospective data

was no longer available as pharmacy records are only kept on file for one year due to storage limitations.

If a physician considers a patient to be particularly stable, they might instruct the pharmacist to permit what are known as 'carries.' This allows the patient to take one or more doses of methadone to ingest unsupervised. Typically, patients are required to pick-up their prescription at least once or twice a week; the most common formats for administering carries are to allow carries on weekends or to allow biweekly pick-up. Physicians typically don't allow methadone carries unless the patient is very stable and there are usually only a few eligible patients receiving carries at any given time.

The pharmacy records are entered directly into a relational database, each record representing one dose administered for a specific patient. On certain days, the methadone prescription may be prepared by the pharmacist but the patient may not show up and the dose may not be administered. In these cases, the doses are recorded as "Not Filled." Furthermore, a physician may cancel a prescription by phoning the pharmacy, in which case the dose is recorded as "Reversed." Additionally, sometimes patients require an extra dose on a day, because they have vomited the original dose or require a dose adjustment. In these cases, multiple doses for a patient are recorded.

Patients are assumed to have received their methadone dose for a given day if a record exists indicating that the dose was "Filled." If no record exists, patients are assumed to have missed their dose. This data is checked against the physician's instructions to determine if patients were eligible to receive carries. If carries were permitted, patients were excluded from

consideration, until the instructions made no mention of carries. This is to ensure methadone adherence is being measured appropriately and is a suitable surrogate for HAART adherence.

2.3 Urinalyses

In Chapter 3, we want to assess the impact of ongoing illicit drug use, as measured by urinalyses results, on methadone adherence. Urinalyses are done to monitor illicit drug use as part of the monitoring requirement of the clinic's methadone maintenance program. Guidelines usually indicate testing should be done on a regular (approximately every 14 days), but not predictable basis, so that patients aren't able to disguise their drug use. Although testing can be done for a battery of different illicit drugs, only amphetamines, benzodiazepines, cocaine and opiates are routinely tested for at the clinic. Urine samples are collected onsite at the clinic and sent to a local certified reference laboratory where they undergo a serial testing procedure. Initial testing is done using the COBAS INTEGRA 800 (Roche Diagnostic Systems, Mississauga, Canada) system. Specimens reactive for opiates, cocaine, amphetamines and benzodiazepines are then retested and confirmed using Gas Chromatography/Mass Spectrometry (GC/MS); this procedure eliminates methadone as a possible opioid. Test results simply indicate whether the patient tested positive or negative for each drug class. This data was collected in July 2005, with all urinalysis results for eligible DOT patients being obtained as far back as September 2002.

2.4 Antiretroviral Drug Histories

Detailed ARV drug histories were created for every patient ever enrolled in the Pender DOT program. Information about treatment type, duration and any deviations from the standard

DOT protocol, such as allowance for weekly or monthly HAART distribution, was sought. The BC Center for Excellence in HIV/AIDS (CFE) in Vancouver maintains a Drug Treatment Program (DTP) database of every patient prescribed ARVs in British Columbia who received a government reimbursement for the cost of such drugs. The CFE makes this data available upon request to BC physicians, as the success or failure of certain drug regimens significantly affect the appropriate choice for subsequent regimens. The DTP database is created from pharmacy records of prescription and prescription refill data and is assumed to be accurate within a month. All patients enrolled in BC's Medical Services Plan (MSP) receive ARVs at no direct cost and drug dispensement is done centrally at the CFE. Typically, prescriptions are submitted by the prescribing physician to the CFE for approval. Once approved, the patient can pick up their prescription from the CFE, or ask that their pharmacist request it be sent to his or her pharmacy. Refills every 1-3 months must be made in a similar fashion but would generally not require approval.

The DTP database may be assumed to be accurate for the general population, but accuracy is problematic for patients enrolled in the DOT program. Once approval has been granted, the staff at the Pender Clinic arrange for the ARVs to be sent to the clinic or directly to the pharmacy. As soon as the ARVs are received, the DTP database assumes that drug therapy has been initiated and will continue for as along as the supply allows. For example, if 90 days of pills were dispensed on June 1st, the patient would be assumed to take their first pill on June 1st and their last pill on August 29th. However, often patients enrolled in a DOT program do not initiate therapy immediately upon receipt of the ARVs or the patient may decide to discontinue therapy prior to the refill date. Moreover, any significant delay of initiating therapy allows for

continuing therapy beyond the time at which the DTP database would assume a patient terminated therapy.

For these reasons, drug histories originating from the DTP serve as a guide to construct drug histories for these patients but are not nearly accurate or complete enough to use alone. Utilization of the chart notes along with consultation with the physicians and nursing staff elucidate the treatment history far more completely. However, even when combining these resources, dates of initiation and termination of therapy are only approximate. Determining the exact dates of initiation and termination is usually not possible. Typically, only the month of initiation and termination of therapy are considered accurate. While many patients take the same regimen consistently over many years, others may change their regimen due to toxicities, or may intermittently start and stop drug therapy. For the former, having approximate initiation and termination dates does not pose a significant problem, but this is not the case for the latter.

While every patient in this study was required to receive methadone daily, not all receive their ARVs with their methadone at the pharmacy. For more stable patients, physicians will often prescribe anywhere from half a week to one month's worth of ARVs in a blister pack. The number of patients in this situation is a small minority, and we have included them in Chapter 4 but excluded them in Chapter 5. Also obtained when collecting ARV histories from the chart notes, was any health care provider noted non-adherence, usually indicating that the pharmacist had reported that the patient either refused to take or did not take their ARV drugs when filling their methadone prescription.

2.5 HIV Plasma Viral Load

The clinic maintains a research database on patients, which includes viral load, CD4, and genotypic resistance testing data. Unless the patient is enrolled in a clinical trial, all HIV plasma viral load measurements are done in the University of British Columbia Virology Laboratory at St. Paul's Hospital in Vancouver. Standard of care in BC is to measure plasma viral load in HIV-positive patients once every 90 days unless otherwise indicated. In a population of IDUs obtaining regular follow-up can sometimes prove problematic, especially during times of significant drug relapse. Hence, the time between two successive HIV plasma viral load measurements for IDUs can be quite variable, with periods as short as a few days and as long as a year.

HIV plasma viral load is measured using the ultrasensitive version of the HIV-1 Monitor assay (Roche Diagnostics, Mississauga, ON). As previously mentioned, HIV plasma viral load is a measurement of the number of copies of viral RNA present once the sample is amplified using PCR. The assay is sensitive between 50 copies/mL and 100,000 copies/mL; tests outside this range are reported as < 50 copies/mL and > 100,000 copies/mL. Typically the therapeutic goal of HAART is called viral suppression, occurring when the viral load is below the detectable limit (in our case < 50 copies/mL). When a new test result is reported, the laboratory also sends a complete history of all HIV plasma viral load test results conducted at the Virology Lab. For some patients, over ten years worth of test results may be available.

2.6 CD4+ Cell Count

CD4+ cell count is another clinically relevant outcome that is measured on a regular basis. Usually whenever a sample is taken to measure HIV plasma viral load, a sample is also obtained to measure CD4+ cell count. CD4+ cell count is measured by flow cytometry at the diagnostic lab located at St. Paul's Hospital in Vancouver. Unlike HIV plasma viral load, historical tests are usually only reported for tests occurring within the last few months. Hence, some of the older tests may have not been collected, as the original test results have been archived. Recent test results are usually complete.

2.7 Drug Resistance Data

Both genotypic and phenotypic drug resistance tests were performed at the British Columbia Center for Excellence in HIV/AIDS using the VirtualPhenotype™ assay. This assay allows for detection of resistant mutants in the plasma when the HIV viral load exceeds 250 copies/mL. The standard of care has evolved over the study period to call for genotypic testing to be performed when any virus is present in the blood. In the past, tests were performed at a much less frequent rate than currently. In both the contemporary and historical cases, the blood sample obtained for the viral load testing is also used for resistance testing. In most cases residual plasma is left-over from the viral load test, and if resistance testing hasn't been ordered, the left-over sample is frozen and resistance testing can be ordered at a later date.

2.8 Study Interval Construction and Approach to Statistical Analysis

In each of the subsequent analyses, a longitudinal approach was undertaken. Time intervals were constructed as the time between two clinical measurements and the methadone adherence was calculated between these events. In Chapter 3, we present data which attempts to relate methadone adherence to the results of urinalyses. For patients enrolled in the DOT program, time periods representing the time between two consecutive urinalysis tests were constructed. The methadone administration database was then queried and intervals without a single methadone administration record were assumed to be periods when the patient was not enrolled in the methadone maintenance program; these were excluded from further analysis. In those intervals where at least one methadone dose was administered, adherence was calculated as the proportion of days where methadone was administered (at least once) during the time period between the two urinalysis results. In certain intervals, the first methadone dose was received some time after the first urinalysis test. We set an arbitrary guideline which excluded intervals in which the patient hadn't received a single methadone dose within one month (30 days) of the interval's first urinalysis result. Furthermore, a few patients had extremely long time intervals where the interval's two urinalysis test were more than a year apart. When this was accompanied by a prolonged period of non-adherence (> 90 days), the patient was assumed to have left the methadone maintenance program and the time interval was excluded from further analysis. Possible explanations may be that the patient moved out of British Columbia or was incarcerated.

In Chapters 4 and 5, intervals are constructed to represent the time between two consecutive viral load measurements. These intervals were intended to be restricted to periods of time where the patients were receiving HAART by DOT. Due to the limitations in the way that

HAART treatment is documented at the clinic, initiation and termination of HAART are only considered to be accurate within one month. In time intervals which occurred during dates where patients were estimated to have started or terminated their HAART regimens, it was often difficult to ensure patients began HAART shortly after the interval's first viral load measurement, or didn't terminate it before the interval was over. Viral loads occurring within the same calendar month where the patient was indicated to have initiated or terminated therapy were assumed to have occurred in close enough proximity to the dates of initiation or termination. When regimens were terminated and either started or changed on consecutive months, continuity of therapy was assumed.

As for Chapter 3, the methadone administration database was queried for methadone doses administered during each of these intervals. Intervals where not a single dose was administered were excluded from further analysis. During each interval, patients were required to receive their first methadone dose within 10 days of the interval's first viral load measurement. Those intervals where methadone was first administered more than 10 days after the interval's first viral load measurement were excluded from further consideration. For all intervals entering into the analysis, methadone adherence was then calculated as the fraction of days where methadone doses were administered during the time period extending from the first methadone dose to the end of interval viral load. This dataset was then used for the analysis in Chapter 4.

In Chapter 5, we considered the subset of intervals from Chapter 4 for which the end of interval viral load exceeded 250 copies/mL. In those intervals, beginning of interval DRMs included all DRMs detected in any previous genotypic resistance test. Emergent resistance was then evaluated by comparing the results of the end of interval genotypic resistance test to the

beginning of interval DRMs. Mutations which were not present at the beginning of interval assessment were counted as emergent DRMs. New DRMs occurring at the same amino acid position within the same gene as a previously detected DRM were not considered as emergent DRMs. The count of these mutations on each of the protease and reverse transcriptase genes were considered separately, as well as the aggregate total of new DRMs in each interval.

The structure of the intervals lends itself nicely to longitudinal data analysis. Since all our hypotheses are framed at the population level and we don't require estimation of individual level data, we have chosen to take a marginal approach to the data analysis. Specifically, we use the generalized estimating equation (GEE) [22] method to estimate the effects of a variety of different predictors using widely used generalized linear models such as Gaussian, logistic and Poisson regression [23]. In all such analysis, the robust standard errors of the parameter estimates were used as a basis for conducting statistical inference.

3 The Impact of Ongoing Illicit Drug Use on Methadone Adherence

3.1 Background

Before undergoing a thorough examination of the impact of HAART adherence measured by methadone adherence on different HIV-related clinical outcomes, it may be important to first examine which factors affect methadone adherence. Studies which have looked at the effectiveness of opiate substitution therapy programs, such as methadone, whether they are in a detoxification or in a maintenance therapy setting, have typically looked at abstinence from psychoactive substances and treatment retention as their primary outcomes [24-29]. Using these outcomes, baseline or ongoing drug use has been demonstrated to be predictive of poor outcomes in drug treatment programs, as well as higher rates of relapse to substance use [30-32].

However, in recent years, methadone has been utilized as a method of engaging patients in other forms of care through programs such as DOT. Specifically, methadone maintenance programs have been coupled with antiviral therapy for the treatment of HIV [17-19, 21] and HCV [33-35] infections as well as with antibacterial therapy for the treatment of respiratory tract and soft tissue infections [36]. In such programs, methadone and other prescribed medications can be co-administered daily by a community pharmacist. In these settings, adherence to therapy is closely tied to adherence to methadone and ensuring high methadone adherence is necessary for successful therapeutic outcomes [5-7, 37]. The specific level and pattern of adherence that is required has not been well defined for any of the medical conditions under treatment. Before this can be done, we need to be certain that methadone adherence itself can be appropriately

characterized in the target population being treated for these conditions. With this in mind, we have evaluated adherence to methadone in patients attending an inner city clinic and further examined what factors may impact on variability in adherence over time.

3.2 Methods

3.2.1 Study Design and Treatment Program

This retrospective longitudinal study was conducted at the Pender Community Health Center, a multidisciplinary clinic located in the Downtown Eastside of Vancouver. Patients were eligible for inclusion in this study if they enrolled in the clinic's directly observed therapy (DOT) program as well as the clinic's methadone maintenance program during the study period, October 2002 to July 2005. The clinic's DOT program couples methadone maintenance therapy with highly active antiretroviral therapy (HAART) administration. Patients are required to present themselves daily at a community pharmacy where they are co-administered methadone and their HAART [17, 18].

Within the clinic's methadone maintenance program, urinalysis for illicit drugs are performed as a component of monitoring patient compliance with the objectives of methadone maintenance therapy. Urinalyses are performed frequently, but at unpredictable intervals as recommended by provincial guidelines for the testing of illicit drugs in urine. Moreover, frequency of testing is often individualized and may occur much less frequently in stable long-term methadone maintenance therapy patients and more often in patients whose illicit drug use is more erratic. Patients are routinely tested for opiates, cocaine, amphetamines, and

benzodiazepines at a local certified reference laboratory using COBAS INTEGRA 800 (Roche Diagnostic Systems, Mississauga, Canada). Specimens reactive for opiates, cocaine, amphetamines and benzodiazepines were then retested and confirmed using Gas Chromatography/Mass Spectrometry (GC/MS).

Daily methadone dose is also individualized to the patient's needs. Doses are frequently titrated within this program to alleviate any opiate withdrawal or to counteract any pharmacokinetic interactions exhibited by several of the antiretroviral drugs [38]. Decreases in dose are often observed in patients wanting to reduce their daily requirements of methadone or those who are discontinuing antiretrovirals known to interact pharmacologically with methadone absorption or metabolism.

3.2.2 Data Collection

All urine drug screen tests were collected for each eligible patient and daily methadone administration data were obtained using pharmacy records. Each daily methadone administration record was then associated with the closest urine drug screens before and after the day of administration. In those days which lacked an administration record or when the prescription was not filled, the patient was assumed to have missed their dose. The time periods where no methadone doses were administered between two urine drug screen tests were excluded from further analysis as the patient was assumed to have terminated their methadone maintenance therapy. If, however, the patient re-initiated therapy at a date subsequent to their termination of therapy, these latter time periods were included in the analysis. Furthermore, in patients who

received one or more methadone doses for administration outside the pharmacy, these time periods were also excluded as daily methadone adherence was not measurable.

Urinalysis results for each illicit drug class (amphetamines, benzodiazepines, cocaine, and non-methadone opiates) are reported as being positive or negative. Furthermore, the methadone dose administered on each day was recorded. If a patient did not have a methadone administration record for a given day (i.e. they missed a dose), the most recent dose was used (78 missed doses were imputed). The most recent previous and subsequent urinalyses were associated with each study day for each patient. In addition to these time-related urinalysis data, baseline data including age and sex were collected.

3.2.3 Statistical Analysis

The impact of illicit drug use on methadone adherence was examined longitudinally. For each patient, time intervals were created designating the time between two successive urinalysis tests. Methadone adherence was computed as the percentage of days where methadone had been administered during the time interval between the two urinalysis tests. Illicit drug use was defined in two different ways. First, illicit drug use for each class was defined as testing positive at either the beginning or the end of the time interval. This allows one to assess the impact of illicit drug use at anytime during the time interval. Second, illicit drug use at the beginning and end of the interval were considered separately. This allows one to evaluate the possibility of an initial urinalysis being useful in predicting subsequent adherence.

Levels of adherence were first described separately for positive and negative intervals for each drug class. Linear regression models were used to determine the effect of illicit drug use and other variables of interest on methadone adherence. To account for the correlated nature of the repeated measurements on each patient, the generalized estimating equations (GEE) method [22] was used with an exchangeable correlation structure to fit all of the linear regression models.

To assess the impact of individual predictors on the levels of adherence, separate univariate linear regression models were first fit for each drug class, and for sex, baseline age, time on study, and methadone dose on the day of the interval's first urinalysis test. Methadone dose was considered separately as a continuous and categorical predictor, with dose categories <50 mg/day, 50-100mg/day, 100-150mg/day, and >150mg/day.

Subsequently, each illicit drug class, along with the other potential confounders were included in a multiple linear regression analysis. Two term interactions between each of the illicit drug classes were of interest to assess if the effects of using multiple illicit drug classes were non-additive. After fitting a model incorporating all the main effects and the two term interactions between illicit drug classes, backwards elimination was used to simplify this multiple regression model.

In a separate analysis, urinalysis results for illicit drug use at both the beginning and end of each interval were incorporated into a multiple linear regression model, along with all potential confounders. Such a model would assess the utility of using an initial urinalysis result to predict subsequent adherence. Two term interactions between illicit drug classes at the same time point

were also included in the initial model fit. Model selection was then conducted in a similar manner as above.

3.3 Results

A total of 67 patients were screened for eligibility for this study, with 7 being excluded. Five patients had not received a single methadone dose during the study period, while two others had prescription records indicating that methadone was administered bi or tri-weekly, which did not allow for direct measurement of methadone adherence. The characteristics of the remaining 60 patients are summarized in Table 3.1.

Table 3.1. Patient Characteristics

N	60
Female	21 (35.0%)
Male	39 (65.0%)
Mean Follow-up (days, SD)	341 (212)
Mean Baseline Age (years, SD)	39.2 (6.4)
Mean Methadone Adherence (all follow-up, SD)	84.5% (20.0%)
Mean Daily Methadone Dose (mg/day, SD)	88.5 (56.8)
Median Number of Urinalysis Tests (IQR)	8 (5.3)
Median Length of Time Between Urinalysis Tests (IQR)	28 (37.8)
All Urinalysis Results Negative (for all drugs, at every study test)	4 (6.7%)
Positive Amphetamine Test (at any time during study)	28 (46.6%)
Positive Benzodiazepine Test (at any time during study)	37 (61.7%)
Positive Cocaine Test (at any time during study)	54 (90.0%)
Positive Opiates Test (at any time during study)	47 (78.3%)
Positive Urinalysis Results for 2 or More Different Drug Classes*	51 (85.0%)
Positive Urinalysis Results for 3 or More Different Drug Classes*	33 (55.0%)
Positive Urinalysis Results for 4 Different Drug Classes*	8 (15.0%)

*On the same urinalysis test.

A total of 20,479 days were observed, with 18,106 methadone doses administered during the study period for an overall adherence of 88.4%. Of the 488 intervals, 217 (44.5%), 294 (60.2%), 334 (68.4%), and 393 (80.5%) intervals had methadone adherence of 100%, > 95%, > 90% and > 80% respectively (Figure 3.1). High rates of illicit drug use were observed during the study, with only 4 patients (6.7%) abstaining from all illicit drug use throughout the entire study. Of the 548 urinalysis results used in this analysis, cocaine and opiates were the most frequently detected drugs with 300 (54.7%) and 178 (32.5%) positive results (Table 3.2). Poly-substance use was common, with 51 patients (85.0%) testing positive for two or more illicit drug classes in the same urinalysis at some point during the study (Table 3.1). Cocaine and opiates were the most frequently occurring combination with 138/548 (25.2%) of the urinalysis test results indicating their simultaneous presence (Table 3.2).

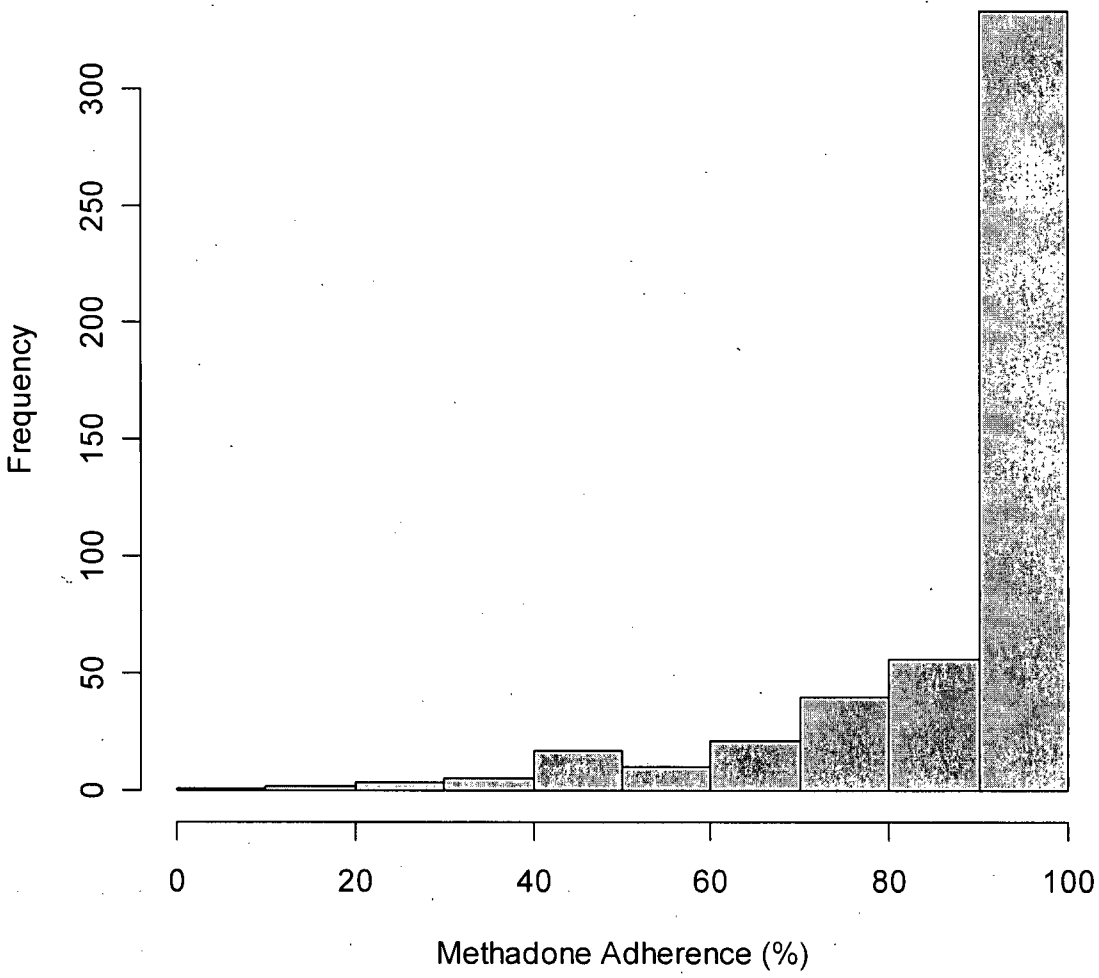

Figure 3.1. Distribution of methadone adherence observed in the time intervals

Table 3.2. Urinalysis Test Result Distribution

Class of Illicit Drug	Number of Positive Urinalysis Results (N=548)
Amphetamines	70 (12.8%)
Benzodiazepines	162 (29.6%)
Cocaine	300 (54.7%)
Opiates	178 (32.5%)
Amphetamines + Benzodiazepines	16 (2.9%)
Amphetamines + Cocaine	46 (8.4%)
Amphetamines + Opiates	30 (5.5%)
Benzodiazepines + Cocaine	131 (23.9%)
Benzodiazepines + Opiates	63 (11.5%)
Cocaine + Opiates	138 (25.2%)

As would be expected, mean adherence was much higher in intervals in which no evidence of opiate use was documented (both urinalysis results negative for opiates), with a mean 93.8% adherence in intervals where opiates were not detected and 84.2% in those intervals where opiates were detected. Mean adherence was also lower in those intervals where amphetamines were detected, but to a much lesser extent. However, slightly higher mean rates of adherence were noted in intervals when benzodiazepines or cocaine were used (Table 3.3).

Table 3.3. Mean Methadone Adherence by Drug Use

Class of Illicit Drug	Intervals with a Positive Test*		Intervals with a Negative Test*	
	Number	Mean Adherence (SD)	Number	Mean Adherence (SD)
Amphetamines	93	88.0% (19.7%)	395	89.7% (16.0%)
Benzodiazepines	207	91.1% (14.4%)	281	88.2% (18.2%)
Cocaine	319	89.6% (16.3%)	169	89.1% (17.7%)
Opiates	221	84.2% (20.3%)	267	93.8% (11.5%)
Amphetamines + Benzodiazepines	35	83.9% (21.7%)	453	89.8% (16.3%)
Amphetamines + Cocaine	69	85.4% (21.7%)	419	90.1% (15.7%)
Amphetamines + Opiates	51	83.0% (23.1%)	437	90.2% (15.7%)
Benzodiazepines + Cocaine	181	90.7% (14.7%)	307	88.6% (17.8%)
Benzodiazepines + Opiates	110	87.3% (17.2%)	378	90.0% (16.6%)
Cocaine + Opiates	181	85.9% (19.0%)	307	91.5% (14.9%)

*Positive meaning positive at either the most recent previous urinalysis or the most proximate upcoming

Possible relationships between methadone adherence and several covariates were first explored graphically. In Figure 3.2, boxplots of methadone adherence by urinalysis result (positive indicating that the patient tested positive at either interval urinalysis test) are presented. Relationships between methadone adherence and baseline age, sex and methadone dose are explored in Figure 3.3.

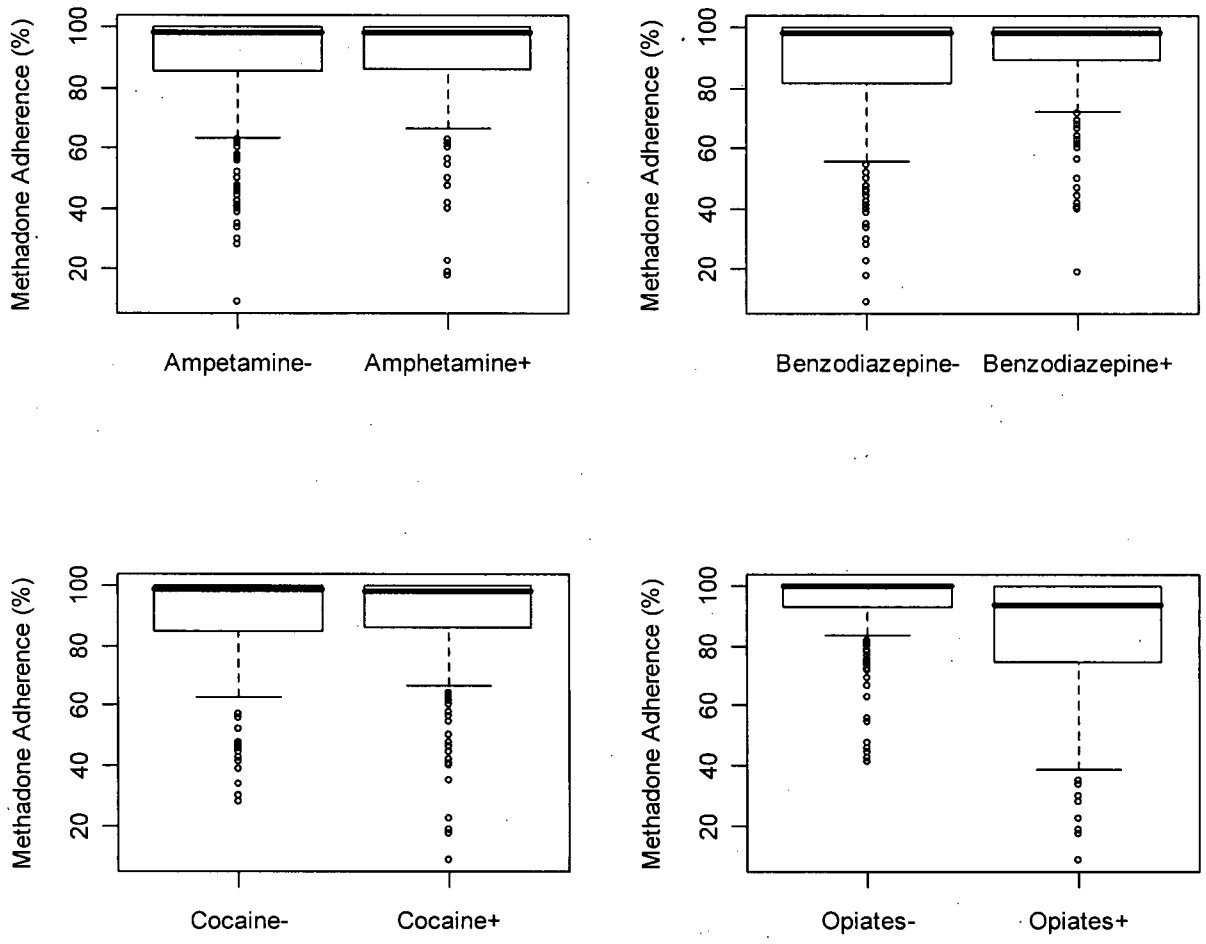

Figure 3.2. Methadone adherence by urinalysis status for each class of illicit drug

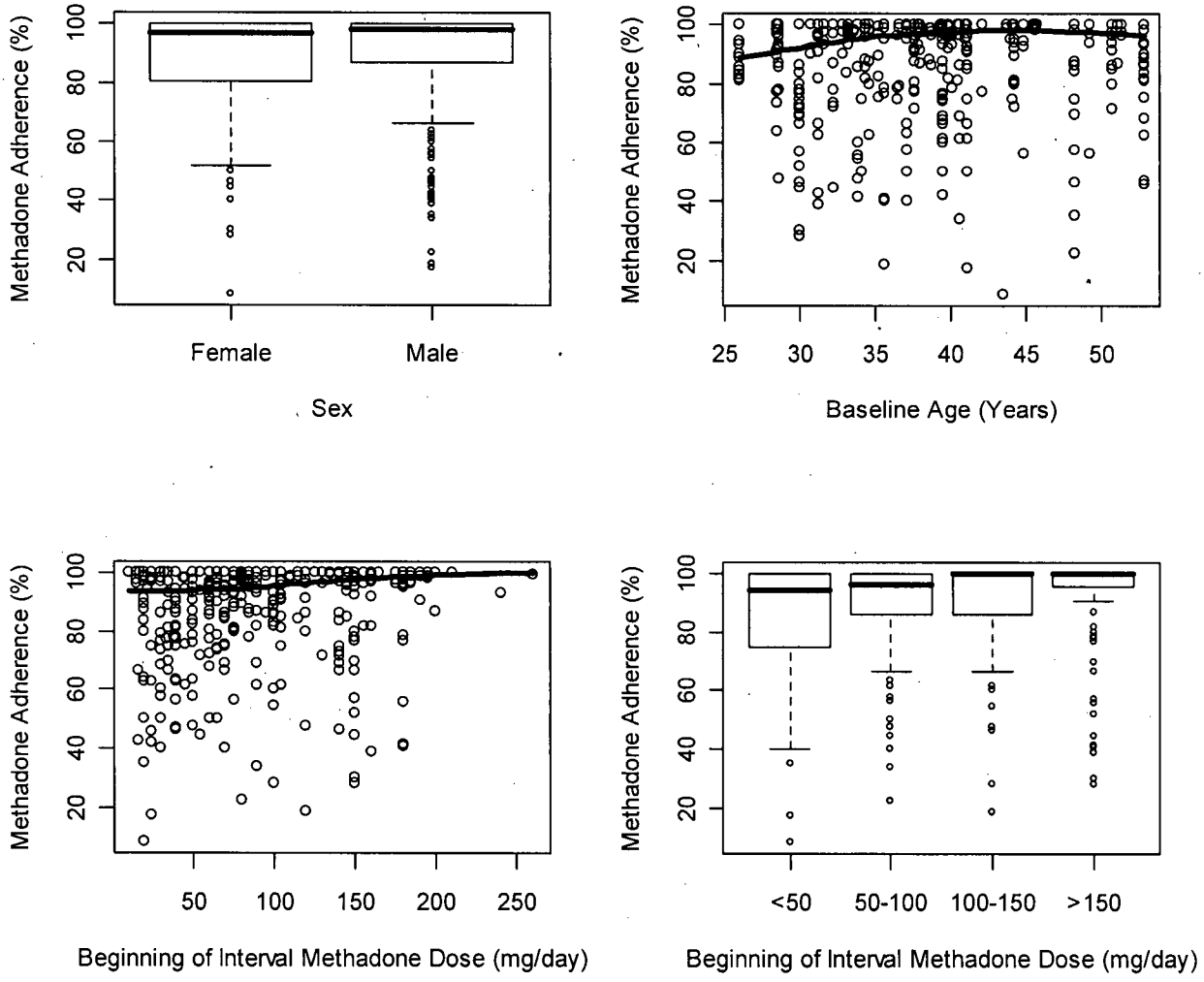

Figure 3.3. Methadone adherence versus several possible predictors. The lines in the upper right and lower left plots were computed using the lowess smoother.

Exploration of the correlation structure of the repeated adherence responses revealed that the correlation between intervals on the same patient did not appear to decrease as a function of time separation. The correlations were relatively strong (r = 0.2-0.8), but there was considerable variability among the calculated correlations at different lags. As neither independence nor

autoregressive working correlation structures would yield efficient GEE analyses, an exchangeable working correlation structure was chosen for the following linear regression analyses.

Under univariate analyses (Table 3.4), among the illicit drug classes, only opiates had a statistically significant impact on methadone adherence, with an estimated decrease in adherence of 7.8% (95% CI: 3.9%-11.7%, p-value<0.001). Daily methadone dose, when considered as a continuous predictor, was associated with higher rates of adherence (+1.0% per 10 mg/day increase in dose, 95% CI: 0.4%-1.6%, p-value<0.001). Similarly, when daily methadone dose is considered as a categorical predictor, increased rates of adherence are estimated when comparing 50-100mg/day (+5.9% 95% CI: 0.6%-11.3%, p-value=0.029), 100-150mg/day (+8.5%, 95% CI: 2.2%-14.9%, p-value=0.009), and >150mg/day (+9.3%, 95% CI: 0.0%-18.7%, p-value=0.051) to <50mg/day.

Table 3.4. Univariate Regression Analyses of Factors Affecting Methadone Adherence

Factor	Estimated Effect on Adherence	95% Confidence Interval	p-value
Amphetamines	-3.1%	(-7.9, 1.8)	0.21
Benzodiazepines	-0.4%	(-4.3, 3.5)	0.86
Cocaine	-2.4%	(-6.4, 1.6)	0.25
Opiates	-7.8%	(-11.7, -3.9)	<0.001
Sex (M vs. F)	+0.8%	(-6.3, 7.9)	0.82
Baseline Age (per 10 year increase)	+1.9%	(-2.5, 6.4)	0.39
Time on Study (per 100 day increase)	-0.5%	(-1.4, 0.43)	0.31
Methadone Dose (per 10 mg/day increase)	+1.0%	(0.4, 1.6)	<0.001
Methadone Dose (relative to <50mg/day)	-	-	-
50-100mg/day	+5.9%	(0.6, 11.3)	0.029
100-150/mg/day	+8.5%	(2.2, 14.9)	0.009
>150mg/day	+9.3%	(0.0, 18.7)	0.051

A multiple linear regression analysis with an initial model incorporating all two term interactions between illicit drug classes and the other covariates was then conducted. A joint Wald test assessing if any of the model's interaction terms were non-zero was statistically significant (p-value<0.001). Therefore, we proceeded with backwards elimination focusing on the interactions first, then on the main effects. If an interaction was marginally significant, it remained in the model until all negligible main effects were eliminated, at which time it was removed if non-significant. This led to the elimination, in order, of benzodiazepines x opiates, amphetamines x opiates, benzodiazepines x cocaine, time on study, patient sex, baseline age, and cocaine x opiates.

The amphetamines x benzodiazepines (p-value=0.008) and amphetamines x cocaine (p-value=0.040) interactions were retained in the final model; see Table 3.5. Opiate use was

associated with decreased methadone adherence of 7.7% (95% CI: 3.8%-11.5%, p-value<0.001). Amphetamine use in the absence of benzodiazepines and cocaine was associated with an increase in methadone adherence (+8.0%, 95% CI: 4.4%-11.6%, p-value<0.001). Benzodiazepines, when used without amphetamines and cocaine, were associated with a small increase in adherence (+3.3%, 95% CI: 0.3%-6.3%, p-value=0.033). However, benzodiazepines or cocaine together with amphetamines negated any of amphetamine's apparent positive effect on adherence. Lastly, cocaine, when used without amphetamines and benzodiazepines, did not significantly affect methadone adherence, while increasing the daily methadone dose appeared to increase adherence slightly.

Table 3.5. Multiple Regression Analysis of Factors Affecting Methadone Adherence

Factor	Subgroup of Intervals	Effect on Adherence	95% Confidence Interval	p-value
Amphetamines use	With No Cocaine and No Benzodiazepines	+8.0%	(4.4, 11.6)	<0.001
	With Benzodiazepines and No Cocaine	-1.9%	(-9.5, 5.8)	0.63
	With Cocaine and No Benzodiazepines	+1.7%	(-4.0, 7.4)	0.55
Benzodiazepines	With No Amphetamines and No Cocaine	+3.3%	(0.3, 6.3)	0.033
Cocaine	With No Amphetamines and No Benzodiazepines	+1.5%	(-2.4, 5.4)	0.46
Opiate Use	No Restrictions	-7.7%	(-11.5, -3.8)	<0.001
Methadone Dose (per 10 mg/day increase)	No Restrictions	+0.8%	(0.3, 1.3)	<0.001

Although GEE does not rely on all of the assumptions required for regression analyses (e.g. normality of residuals), it would be reassuring if these were reasonable. For the final model presented in Table 3.5, a Q-Q normal plot and a scatterplot of the residuals versus the fitted

values is presented in Figure 3.4. The Q-Q plot suggests modest deviations from the normality assumption (driven mainly by ceiling effects) and the residuals versus fitted value plot reveals the 100% constraint on observed adherence.

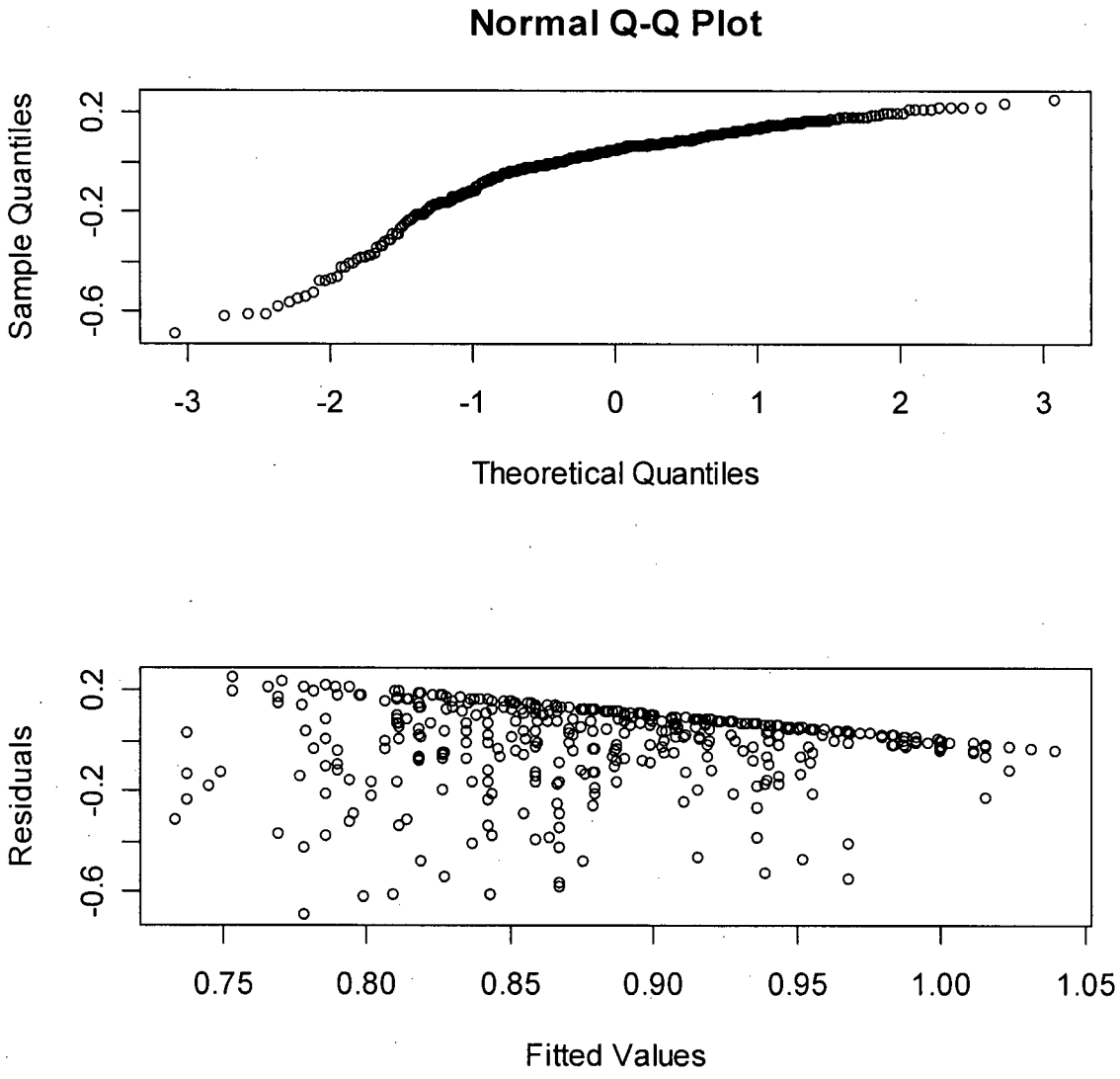

Figure 3.4. Q-Q normal plot and scatterplot of residuals versus fitted values for the model presented in Table 3.5

A multiple linear regression analysis was then performed where the results from each of the two urinalyses associated with each interval were used as separate predictors. Similar to the previous analysis, a joint Wald test was performed to assess if any of the model's interaction terms were non-zero (p-value=0.003). The model selection method was identical to that outlined for the previous analysis. The first phase of model reduction focusing on the interaction effects led to the elimination of all two term interactions between illicit drug classes at the same time point except cocaine x benzodiazepines (beginning of interval) and benzodiazepines x amphetamines (beginning of interval). The second phase of model reduction allowed considerable additional simplification, leading to the final model presented in Table 3.6. The diagnostic plots for this fit appear in Figure 3.5.

Amphetamine and benzodiazepine results from the urinalysis at the beginning of the interval were found to be important, where again a statistically significant interaction was detected (p-value=0.027). Amphetamine use in the absence of benzodiazepines at the beginning of the interval was associated with a modest but statistically nonsignificant increase in methadone adherence. However, when patients were using benzodiazepines, amphetamine use at the beginning of the interval was associated with a decrease in methadone adherence (-7.9%, 95% CI: (-16.1%)-0.2%, p-value=0.056). Opiate use, when detected at the end of the interval, was associated with a decrease in methadone adherence (-4.9%, 95% CI: (-8.4%)-(-1.4%), p-value=0.006).

Table 3.6. Multiple Regression Analysis of Factors Affecting Methadone Adherence With Interval Urinalysis Results Considered as Separate Predictors

Factor	Subgroup of Intervals	Effect on Adherence	95% Confidence Interval	p-value
Amphetamine Use at Beginning of Interval	Without Benzodiazepines	+2.4%	(-1.97, 6.85)	0.28
	With Benzodiazepines	-7.9%	(-16.08, 0.21)	0.056
Benzodiazepine Use at Beginning of Interval	Without Amphetamines	-0.0%	(-3.66, 3.63)	0.99
Opiate Use At End of Interval	No Restrictions	-4.9%	(-8.37, -1.39)	0.006
Methadone Dose (per 10 mg/day increase)	No Restrictions	0.9%	(0.37, 1.41)	<0.001

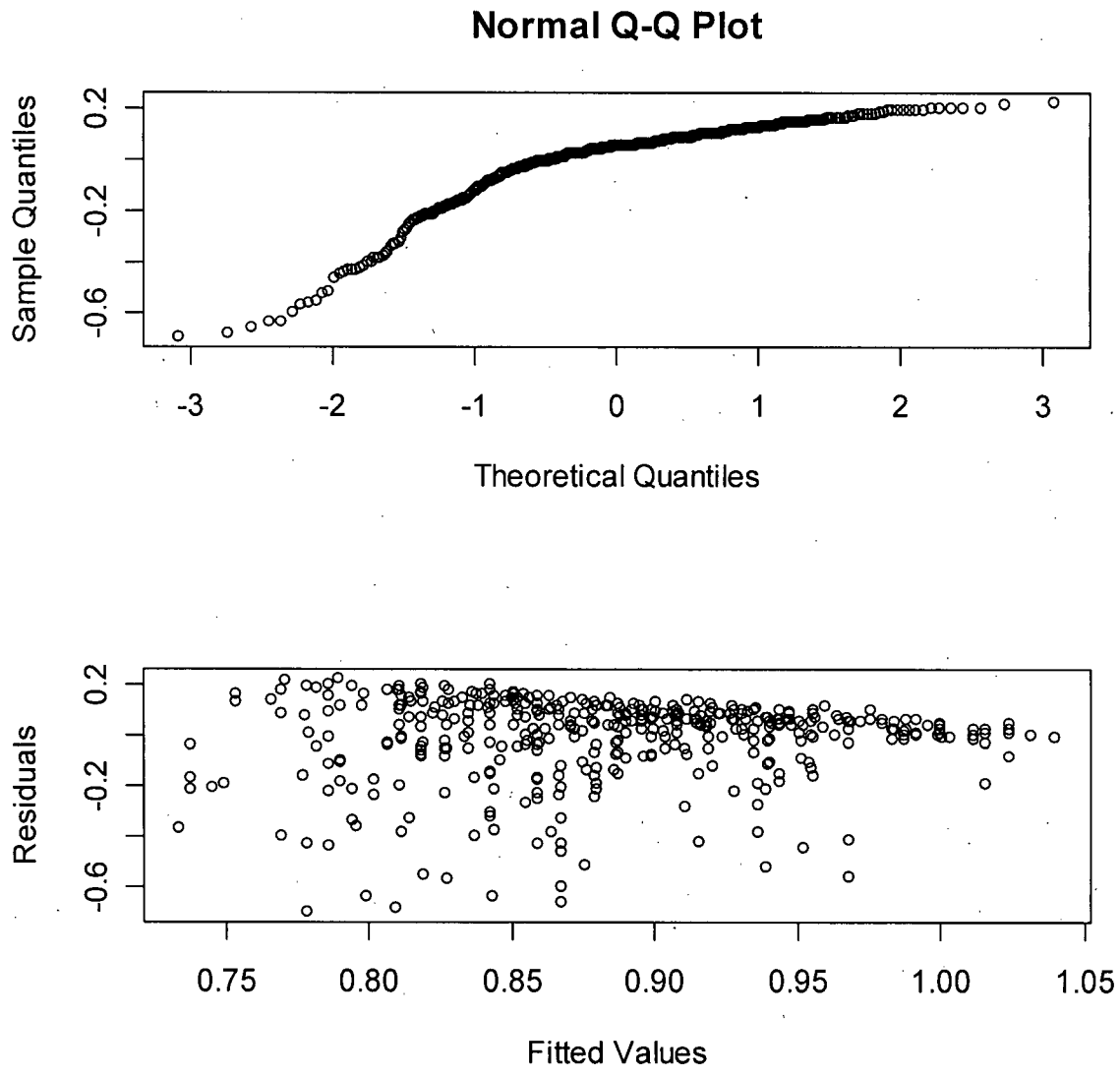

Figure 3.5. Q-Q normal plot and scatterplot of residuals versus fitted values for the model presented in Table 3.6

3.4 Discussion

In subjects enrolled in a long-term methadone maintenance program, high rates of illicit drug use and poly-substance use were detected using routine, regular urinalysis results. Despite

this, high rates of methadone adherence were observed, with over 60% of intervals having rates of adherence that would be suitable for treatment of HIV-infection with HAART (>95%) [5-7] and over 80% of intervals having rates of adherence exceeding those which have been suggested for the treatment of HCV infection with pegylated interferon and ribavirin (>80%) [37].

In our analysis, use of certain illicit drugs does seem to significantly impact methadone adherence. In particular, opiate use (primarily heroin) was observed to significantly decrease methadone adherence by nearly 8% when detected in an interval where either urinalysis tested positive and by nearly 5% when the urinalysis at the end of the interval tested positive. This is not surprising, since methadone is a μ-opioid agonist and relapsing to opiates would require discontinuation of methadone in order to realize the opiate's effects. A positive urinalysis for opiates at the end of the interval likely identifies patients who are actively relapsing in their drug use throughout their interval. It also likely represents individuals who are non-compliant with either pharmacy or medical visits, the latter required every two weeks to renew methadone prescriptions. If patients do not attend these medical appointments, they may be penalized by having their methadone doses held or reduced, leading to further non-adherence with the medical program in the setting of a relapse in drug addiction.

Amphetamine use does seem to affect methadone adherence, but its effect is modified when taken with benzodiazepines or cocaine. Surprisingly, use of amphetamines when taken without benzodiazepines or cocaine was strongly associated with higher methadone adherence. Although this finding was based on a relatively small number of intervals (n=21), there is currently little data in the literature evaluating the effect of baseline or ongoing amphetamine use in isolation on methadone maintenance treatment outcomes. Within animal models, there are

some data indicating that when methamphetamine is co-administered with methadone, increased levels of analgesia are present [39]. Furthermore, in monkeys who were long-term methadone users, when forced to stop methadone therapy, administration of amphetamines acutely produced symptoms that may be consistent with or mimic opiate withdrawal, such as dyskinesia [40]. Either of these effects may increase rates of methadone adherence and are possible explanations for our observations.

However, when amphetamines are taken with benzodiazepines, any positive effect of amphetamine use is negated. Epidemiologic studies have demonstrated that those methamphetamine users who use benzodiazepines are more likely to be injectors of methamphetamine and are also more likely to have concurrent psychosocial problems [41]. Similarly, in this study, the positive effect associated with amphetamine use on methadone adherence was eliminated in intervals where cocaine and amphetamines were taken together. Although little data are available in the literature on amphetamine-cocaine poly-substance use, such patients may exhibit many of the same destabilizing factors as patients using amphetamines and benzodiazepines. Thus patients who use amphetamines and either benzodiazepines or cocaine simultaneously are likely less stable and prone to non-adherence to any program, including methadone maintenance. This is consistent with our finding that combined use of amphetamines with benzodiazepines or cocaine is predictive of decreased adherence when compared to amphetamine use alone. The fact that urinalysis results occurring before a period of methadone therapy are more predictive than those occurring after this period, may help us identify patients in whom specific intervention is required to ensure success of the methadone maintenance program.

Benzodiazepines taken alone were observed to increase methadone adherence. Benzodiazepine use has been previously shown to have both beneficial and harmful properties on substitution therapy outcomes [29, 42]. This appears to be dependent on the context of their use, as abusers of benzodiazepines are hypothesized to have poorer outcomes whereas those who are being treated or self-medicated for psychosocial problems may have better outcomes [43]. This is consistent with our findings, noting that benzodiazepines may be prescribed in some patients and are not a marker of illicit drug use in itself. If they are obtained through illicit means, they may also be used to combat some of the side effects of opiate withdrawal and actually reduce illicit drug use and thereby increase adherence.

In all our analyses, cocaine use by itself was not observed to affect methadone adherence. Cocaine use has been shown previously to be associated with poor outcomes in opiate substitution therapy programs and higher rates of relapse to opiate use [30-32]. However, in our assessment, its impact on methadone adherence was negligible. This may relate to the fact that we are using a different treatment outcome, or that patients considered in this analysis are predominantly long-term heroin users and are thus not representative of cocaine users as generally considered in the medical literature.

This study has several limitations. Firstly, the study population may not be generalizable to all patients receiving methadone maintenance therapy. Patients enrolled in this HIV DOT program have had substantial pre-treatment counselling on the importance of adherence to HAART. Counselling indicated that non-adherence to HAART is associated with high rates of treatment failure [5-7] and, more importantly, is associated with the development of HIV drug resistance which can make subsequent treatment much more difficult [9, 14]. Secondly, the

measurement of drug use is somewhat crude as urinalysis tests do not accurately capture drug use frequency or intensity, both of which may play important roles in affecting methadone adherence.

Therapeutic and reimbursement guidelines often include illicit drug use as a contraindication for the treatment of a number of life-threatening medical conditions, such as HIV and HCV infections. However, the success of the DOT program at our [17, 18] and other [19, 21] centers indicate that drug abstinence is not always required for successful long-term therapeutic interventions, including the treatment of HIV infection. The key to our early success had been the co-administration of methadone with other prescribed medications at community pharmacies that our patients habitually attend. Within this patient population, based upon estimates of methadone adherence, mean HAART adherence would approach 90%, which is in fact substantially higher than those rates of adherence typically observed in non-IDU populations, usually around 70% [5].

Our current analysis helps identify patients to whom special attention should be given to enhance adherence on an ongoing basis. For patients who are using amphetamines and benzodiazepines or amphetamines and cocaine simultaneously, counselling and other interventions should be strongly considered before initiating long-term treatment of HIV infection or other conditions. Similarly, specific attention should be paid to those with ongoing opiate use, especially if they are on a relatively lower dose of methadone. This may help us identify individuals who are not well enough engaged in our programs to benefit from other medical interventions in an optimal way.

A number of programs have made great strides in the successful treatment of HIV and HCV-infected illicit drug users. However, this remains a difficult area of research and medical practice. It must be emphasized that long-term medical treatment programs should not be initiated in all such patients in an indiscriminate manner. Data such as we present in this paper will help identify individuals who are at the highest risk of non-adherence and for whom additional measures specifically directed at their substance use must be undertaken before other complex, life-threatening conditions can be properly and productively addressed.

4 HAART Adherence Thresholds in Maintenance and Induction Therapy Required to Maintain and Achieve Virologic Suppression

4.1 Background

Previous studies have consistently suggested that adherence to HAART < 95% is associated with high rates of virologic failure among HIV-infected patients [5-8, 44, 45]. However, recent data using well-characterized measures of adherence in patients with contemporary HAART regimens of NNRTIs or ritonavir boosted PIs have been largely anecdotal. In fact, two recent studies indicated that the 95% adherence threshold may not apply to such regimens [46, 47].

Typically, studies examining the relationship between HAART adherence and virologic failure have taken a simple cross-sectional approach, where adherence is measured over the entire study period and used as a predictor of virologic failure at a single distant endpoint, the end of follow-up of the study [5-8, 44, 45]. Such cross-sectional studies are limited by their inability to detect periodic lapses in adherence [48], where the overall adherence rate over the study's entire follow-up may not be representative of all dynamic changes in adherence over shorter time periods, which may be the determining factors in the maintenance of long-term virologic suppression. Using a longitudinal study design allows for a more complete appraisal of HAART adherence, as temporal variability in adherence within each patient can be more localized to the outcome of interest.

Further evidence of the need to evaluate adherence in a more insightful manner is provided by a recent report suggesting that for NNRTI-based therapies, repeated unplanned drug cessation (> 48 hours) is an independent risk factor for virologic failure [49]. Conversely, a recent study evaluated the efficacy of a simplified treatment strategy of a five days-on, two days-off dosing schedule in patients on HAART with maximal virologic suppression. Although neither randomized nor controlled, this study showed that 100% of patients taking NNRTI-based therapy maintained virologic suppression through 48 weeks [50]. If adherence < 95% was associated with high rates of virologic failure, as has been previously reported [5-8, 44, 45], this should not have occurred.

To date, no thorough evaluation of potential differences between the adherence-virologic suppression relationship in patients at different stages of treatment has been conducted. Over the past 5 years, we have developed a DOT program for the administration of HAART to IDUs [17, 18]. This allows us to generate information on daily adherence to antiretroviral medications in a longitudinal manner. With this in mind, we have evaluated the thresholds of adherence needed to achieve and maintain virologic suppression in a cohort of individuals receiving contemporary HAART regimens.

4.2 Methods

4.2.1 Study Design and Treatment Program

This retrospective longitudinal study was conducted at the Pender Community Health Center, a multidisciplinary clinic located in the Downtown Eastside of Vancouver. Patients were

eligible for inclusion in this study if they were HIV-infected and enrolled in the clinic's DOT HAART administration program as well as the methadone maintenance program during the study period, October 2002 to October 2005. Within these programs, laboratory testing including CD4+ cell counts and HIV plasma viral load are performed as the standard of care, approximately every three months or as clinically indicated. CD4+ cell count was measured by flow cytometry at the local certified reference laboratory while HIV plasma viral load was measured using the Amplicor HIV-1 Monitor assay (Roche Diagnostics, Mississauga, ON).

Patients selected for this analysis receive HAART and methadone co-administered daily where it is observed by a community pharmacist [17, 18]. Due to regulatory guidelines, daily methadone pharmacy dispensing records are readily available for the entire period of observation and were used as a surrogate for adherence to HAART [51].

4.2.2 Data Collection

All intervals of the time between two successive viral load measurements (usually approximately 3 months) were identified for each eligible patient. Time intervals included in this study were restricted to those where patients were prescribed HAART as indicated by chart notes and prescription records. Intervals where treatment was discontinued prior to the end of the interval (i.e. before the subsequent viral load measurement) or where methadone adherence data was unavailable were omitted from analysis. However, in cases where a patient had eligible and ineligible intervals, all eligible intervals were included in the analysis.

Baseline data including sex, age, antiretroviral (ARV) treatment history, CD4+ cell count, and HIV plasma viral load were recorded at the beginning of each patient's first eligible interval. For each eligible interval, the components of the HAART regimen, HIV plasma viral load at the beginning and end of the interval, and methadone adherence (defined as the fraction of methadone doses consumed during the interval) were determined.

4.2.3 Study Definitions and Outcomes

The primary outcome defining treatment failure was the measurement of HIV plasma viral load above the detectable limit (> 50 copies/mL). Intervals initiated with a plasma viral load below the detectable limit (< 50 copies/mL) were designated as intervals of *maintenance therapy*, whereas intervals initiated with detectable plasma viral load were defined to be intervals of *induction therapy*.

4.2.4 Statistical Analysis

The impact of adherence on the probability of treatment failure was examined longitudinally. To account for the correlated nature of the repeated measurements on each patient, the generalized estimating equation (GEE) method [22] was used with an exchangeable working correlation structure to fit logistic regression models. Estimated HAART adherence, treatment type (NNRTI- or PI-based HAART) and virologic status at the most recent previous viral load measure (i.e. detectable or undetectable) for each eligible interval were considered as predictors of treatment failure. Sex and age at entry to the study as well as time since entry into the study were investigated as additional predictors. Models were first fit separately for each

predictor under univariate analyses, with adherence, treatment type, previous virologic status, and any other statistically significant predictor then incorporated into a multiple logistic regression model. Interactions between adherence, treatment type and previous virologic status were subsequently considered for inclusion in the model; backwards elimination was used to identify any interactions that needed to be included in the final model.

A separate analysis was carried out with adherence expressed as a categorical variable with adherence categories < 70%, 70-80%, 80-90%, 90-95% and ≥ 95% to allow for the evaluation of any important adherence thresholds. The predictors under consideration and final model selection technique followed the procedure as outlined above. Due the high rates of virologic suppression (in a relatively small number of intervals) in the maintenance therapy group with adherence < 70%, it was necessary to combine the < 70% and 70-80% adherence categories when fitting interactions in this analysis.

Additionally, for patients who obtained viral suppression (< 50 copies/mL) at any time during the study, time until virologic failure was determined. Kaplan-Meier survival curves were computed stratifying on overall adherence (calculated from the time of suppression to the time of virologic breakthrough or censoring) greater than versus less than 95%. For patients who obtained viral suppression and subsequently experienced virologic breakthrough followed by re-suppression, only the first chronologic interval was used in this analysis. Failure times were considered censored if patients discontinued therapy prior to documented virologic breakthrough. A logrank test was used to assess the difference in time to virologic failure between the two adherence categories. All reported p-values are two-sided; p-values less than 0.05 were considered statistically significant.

4.3 Results

4.3.1 Patient and Interval Characteristics

Of the 96 patients who received HAART within the clinic's DOT program during the study period, 70 eligible patients contributed a total of 248 time intervals. The primary reason for ineligibility of patients was unavailability or insufficient coverage of methadone pharmacy records due to limited prescribing time during the study period (20 patients). An additional 6 patients were ineligible because they received their methadone prescriptions 2-3 times per week rather than each day, which does not allow for direct estimation of HAART adherence. The mean duration of eligible follow-up was 9.8 months (range, 1 to 29 months), with 59 of the 70 (84.2%) patients observed for more than 3 months. Baseline demographic and clinical characteristics of the 70 eligible patients are reported in Table 4.1. The intervals were on average 3 months in length, which corresponds with the current standard of care. The characteristics of the 248 eligible intervals are described in Table 4.2.

Table 4.1. Baseline Patient Characteristics

N	70
Female	22 (31%)
Male	48 (69%)
Mean Months of Follow-up (SD)	9.8 (7.0)
Mean Age in Years (SD)	40.4 (7.5)
Median Number of Intervals per Patient (IQR)	3.0 (4.5)
Mean Adherence for all Follow-up (SD)	88.1% (13.3%)
Median HIV Plasma Viral Load (copies/mL, IQR)	1105 (53650)
Mean CD4+ Cell Count (cells/mm^3, SD)	245 (181)
ARV Naïve (%)	11 (15.7%)
Baseline Median Drug Experience: # of NNRTIs/ # of PIs / # NRTIs	1/2/3

Table 4.2. Characteristics of Time Intervals

N	248
Therapy Type	
NNRTI-based Therapy	67 (27.0%)
PI-based Therapy	181 (73.0%)
Virologic Status at Start of Interval	
Maintenance Therapy[a]	122 (49.2%)
Induction Therapy[b]	126 (50.8%)
Mean Interval Length (Days, SD)	83.3 (44.0)
Median HIV Plasma Viral Load at Interval Initiation (copies/mL, IQR)	55 (2160)

[a] Intervals with the initial viral load below the detectable threshold (<50 copies/mL)
[b] Intervals with the initial viral load above the detectable threshold

4.3.2 Adherence and Treatment Failure

A total of 18,556 methadone doses were observed by a pharmacist, with 20,666 prescribed over the study period (89.8%, overall adherence). Overall adherence throughout the entire study period was ≥ 95%, ≥ 90%, ≥ 80%, and ≥ 70% in 28/70 (40.0%), 38/70 (54.3%), 57/70 (81.4%), and 65/70 (92.9%) of patients respectively (Figure 4.1). The rate of overall adherence during the study was similar among patients receiving NNRTI-based HAART (90.6%) and PI-based HAART (89.4%).

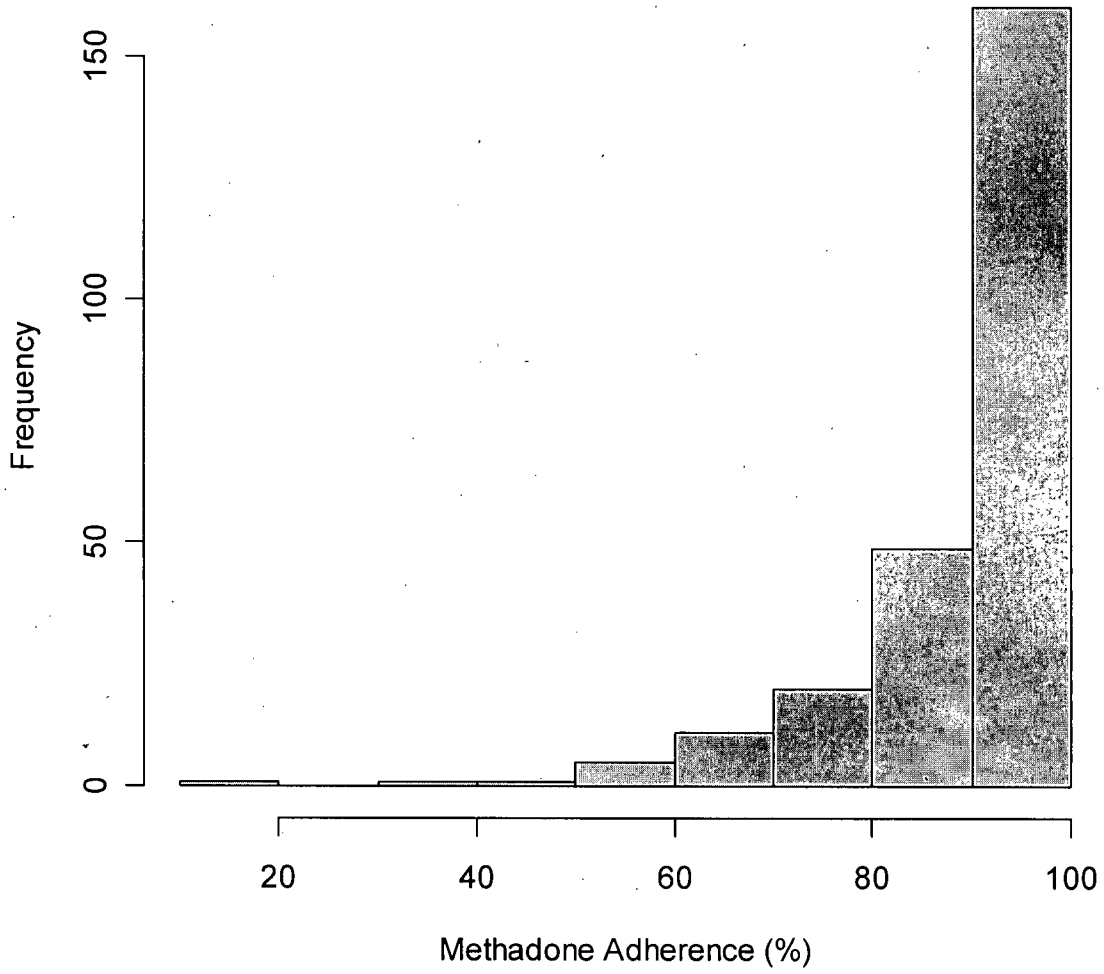

Figure 4.1. Distribution of methadone adherence observed in the time intervals

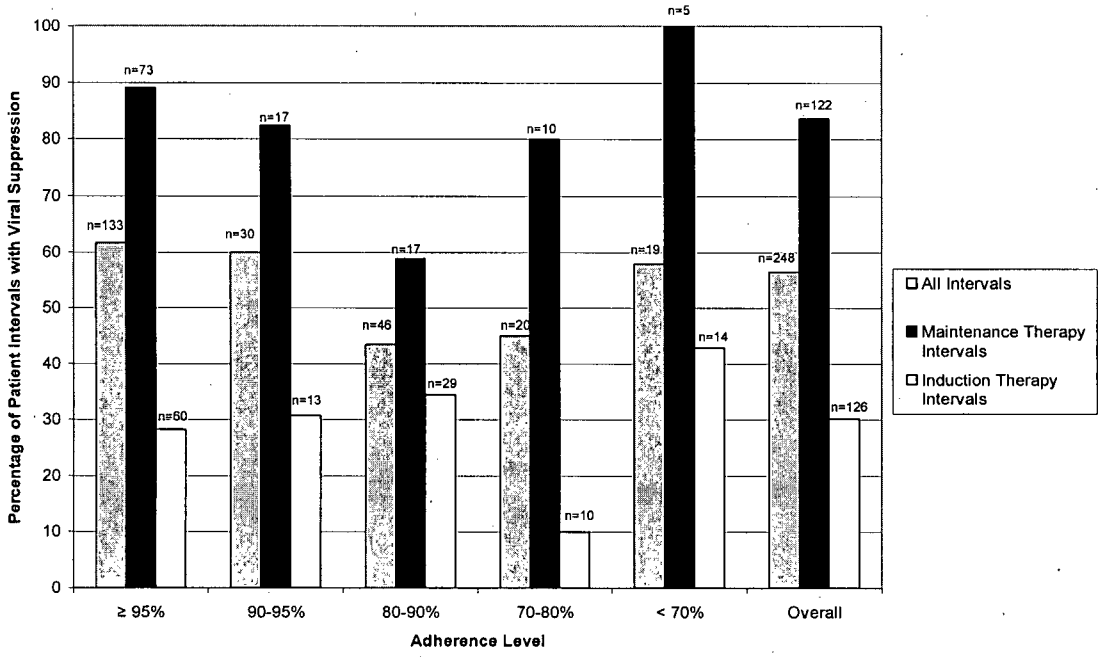

Figure 4.2. Percentage of patient intervals resulting in virologic suppression (< 50 copies/mL) in both maintenance and induction therapy versus level of HAART adherence

The rates of virologic suppression were much higher during intervals of maintenance therapy than during induction therapy (Figure 4.2). Virologic suppression was observed at the end of the interval in 102/122 (83.6%) maintenance and 38/126 (30.2%) induction intervals. In those patients undergoing maintenance therapy, the rates of virologic suppression were consistently high (≥ 80%) over every adherence category, with the exception of the 80-90% category where a higher rate of virologic failure was measured, with 7/17 (41.2%) of the intervals resulting in a detectable viral load. No trend between rates of virologic suppression and adherence level was apparent for the intervals of induction therapy, where rates of viral suppression never exceeded 45%. Figure 4.3 shows no obvious differences between rates of viral

suppression on NNRTI- and PI-based HAART during maintenance therapy. Likewise, no differences were observed during induction therapy (data not shown).

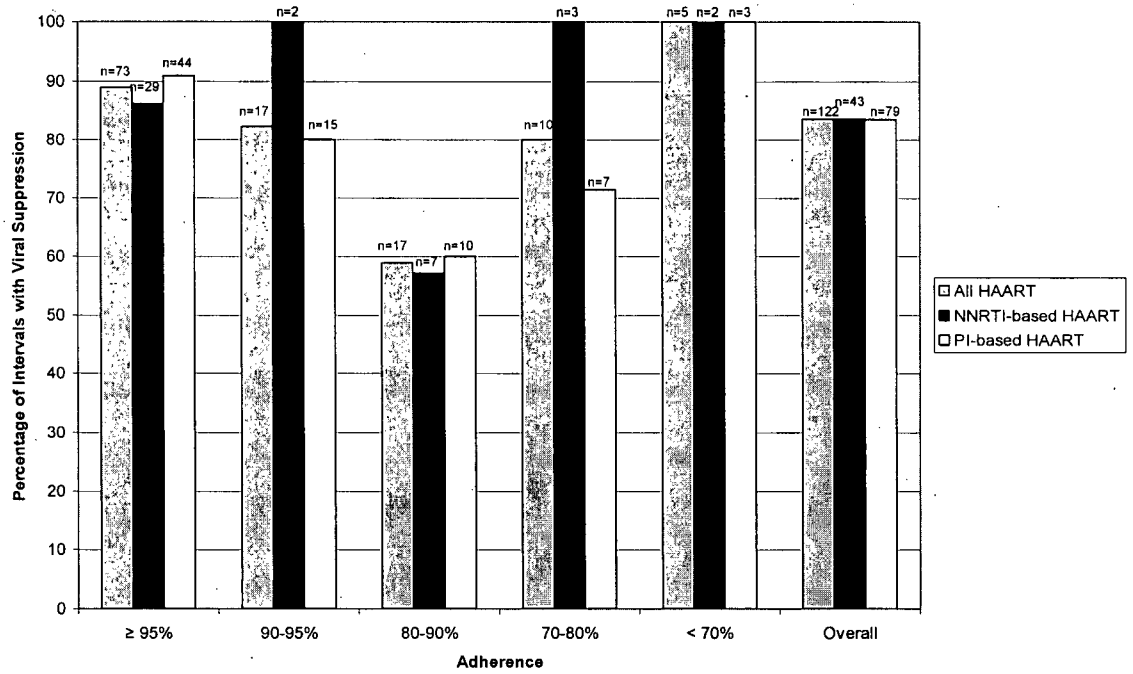

Figure 4.3 – Percentage of patient intervals resulting in virologic suppression (< 50 copies/mL) during maintenance therapy stratified by NNRTI- and PI-based HAART.

Preliminary exploration of the correlation structure of the repeated viral load measurements within individual patients indicated that the correlation between viral load measurements was approximately the same irrespective of time separation. This suggests that an exchangeable working correlation structure is suitable for fitting the logistic regression models.

In univariate analyses, viral suppression at the most recent previous viral load (OR = 9.3, 95% CI: 4.5-18.9, p-value<0.001), baseline age (OR = 1.8 per 10 year increase in age, 95% CI: 1.1-3.1, p-value=0.025), and being male (OR = 2.2, 95% CI: 1.0-4.8, p-value=0.044) were

associated with higher rates of virologic suppression. Adherence whether treated as a continuous or categorical predictor was not associated with viral suppression. Neither treatment type nor time since study entry was associated with viral suppression (Table 4.3).

Table 4.3. Factors Associated with Virologic Suppression in Univariate Analyses

Factor	Odds Ratio	95% Confidence Interval	p-value
Viral Suppression at the Most Recent Previous Viral Load Measurement	9.3	4.5-18.9	<0.001
Adherence as Continuous (per 10% increase in adherence)	0.93	0.77-1.1	0.47
Adherence as Categorical: 90-95%†	1.1	0.52-2.3	0.79
80-90%†	0.72	0.38-1.4	0.32
70-80%†	0.58	0.26-1.3	0.19
< 70%†	1.7	0.52-5.9	0.37
Treatment Type (PI relative to NNRTI)	0.83	0.40-1.7	0.63
Baseline Age (per 10 year increase in age)	1.8	1.1-3.1	0.025
Time Since Study Entry (per year increase)	1.1	0.72-1.8	0.59
Sex (Male relative to Female)	2.2	1.0-4.8	0.044

†Relative to ≥95% Adherence

In a multiple logistic regression where adherence is treated as a continuous predictor, the only statistically significant predictor of virologic suppression at the end of the interval was virologic suppression at the most recent previous viral load measurement (Table 4.4, OR = 9.7, 95% CI: 4.7-20.2, p-value<0.001). When controlling for other predictors, the estimated probability of suppression in an interval actually decreases as adherence increases, although the slope for adherence was not statistically significant (OR = 0.96 per 10% increase in adherence, 95% CI: 0.75-1.22, p-value=0.73). (This is further illustrated in Figure 4.4, where the estimated probability of virologic suppression is predicted to decline as adherence increases after adjusting for virologic status at the beginning of the interval. As in the fit described in Table 4.4, this

estimated decrease was not statistically significant.) No statistically significant difference between PI- and NNRTI-based regimens was observed (OR = 1.2, 95% CI: 0.60-2.5, p-value=0.59). No statistically significant interactions between adherence, previous virologic status, and treatment type were detected, indicating that we have no evidence against the hypothesis that the relationship between our continuous measurement of adherence and virologic suppression is the same in maintenance and induction therapies, and in NNRTI- and PI-based HAART.

Table 4.4. Factors Associated with Virologic Suppression with Adherence as a Continuous Predictor in a Multiple Logistic Regression Analysis

Factor	Odds Ratio	95% Confidence Interval	p-value
Viral Suppression at Most Recent Previous Viral Load Measurement	9.7	4.7-20.2	<0.001
Adherence (per 10% increase in adherence)	0.96	0.75-1.2	0.73
Treatment Type (PI relative to NNRTI)	1.2	0.60-2.5	0.59
Baseline Age (per 10 year increase in age)	1.4	0.92-2.2	0.11
Sex (Male relative to Female)	1.8	0.97-3.3	0.063

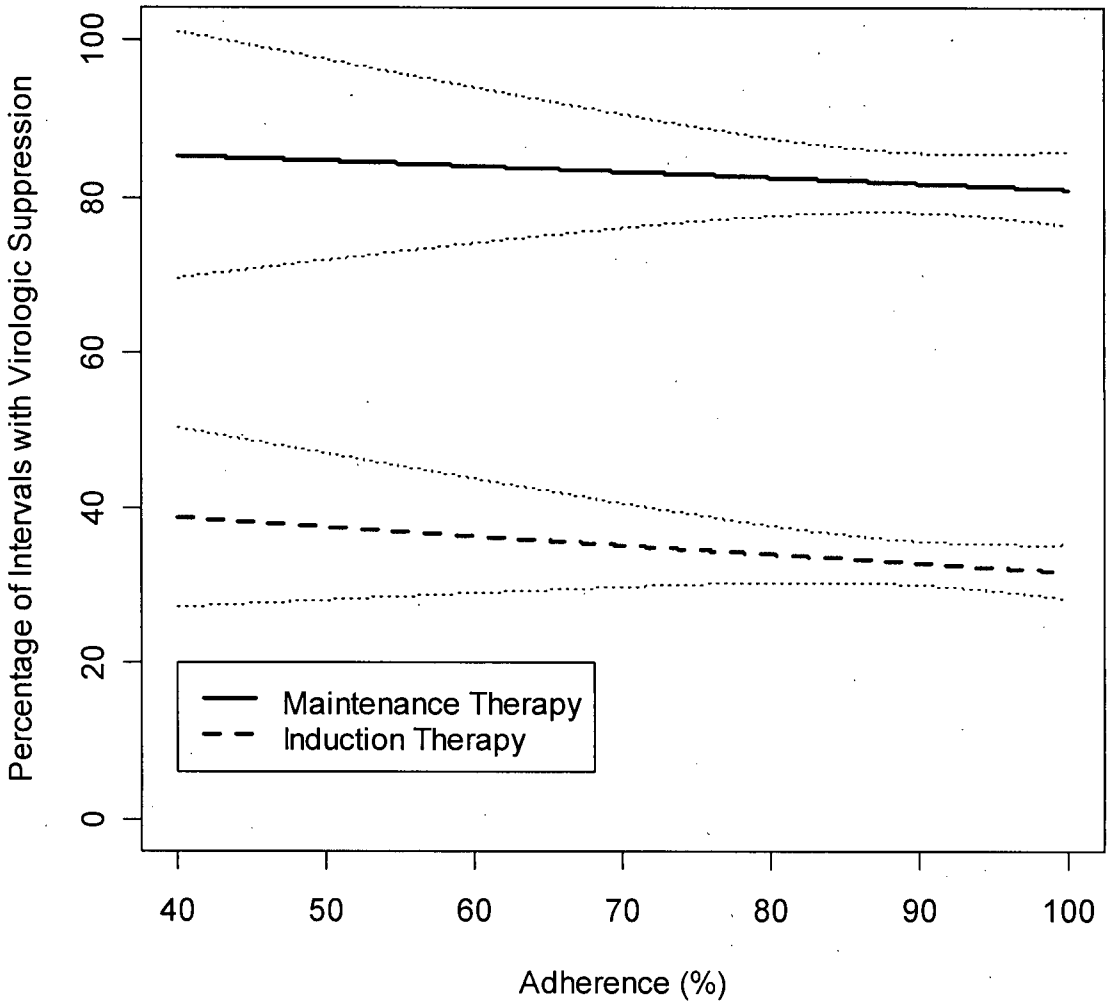

Figure 4.4. Predicted percentage of intervals resulting in end of interval viral load <50 copies/mL) in induction and maintenance therapy. The light dotted lines represent 95% confidence bands.

To examine if any evidence of adherence thresholds existed, multiple logistic regression models were also fit treating adherence as a categorical predictor with adherence categories < 70%, 70-80%, 80-90%, 90-95% and ≥ 95%. A model was first fit assuming that the relationship between virologic suppression and adherence is the same in maintenance and induction therapies

(i.e. without inclusion of any interactions). Under this assumption, no adherence category differed significantly from adherence ≥ 95% (Table 4.5). When interactions were included, only the interaction between adherence and previous virologic status was statistically significant, and the other interaction terms were excluded from further consideration. However, a joint Wald test assessing if any of the model's interaction terms were non-zero was not statistically significant (p-value=0.11). Despite this, an exploratory analysis was then conducted where a model was fit which allowed the relationship between adherence and virologic suppression to be different in maintenance and induction therapies (Table 4.6). In this case, during maintenance therapy only the 80-90% adherence category was estimated to have a lower rate of virologic suppression than the ≥ 95% adherence category (OR = 0.18, 95% CI: 0.05-0.68, p-value=0.011). No statistically significant differences were observed between different adherence categories during induction therapy. For intervals with adherence ≥ 95%, virologic suppression at the most recent viral load measurement was very predictive of virologic suppression when compared to intervals initiated without virologic suppression (OR = 15.6, 95% CI: 5.3-45.7, p-value<0.001).

Table 4.5. Factors Associated with Virologic Suppression with Adherence as a Categorical Predictor in a Multiple Logistic Regression Analysis

Factor	Odds Ratio	95% Confidence Interval	p-value
Viral Suppression at Most Recent Previous Viral Load Measurement	9.6	4.5-20.4	<0.001
Adherence: 90-95%†	0.99	0.38-2.6	0.99
80-90%†	0.71	0.30-1.6	0.42
70-80%†	0.46	0.19-1.1	0.084
< 70%†	1.9	0.51-7.1	0.34
Treatment Type (PI relative to NNRTI)	1.2	0.59-2.4	0.62
Baseline Age (per 10 year increase in age)	1.4	0.85-2.2	0.19
Sex (Male relative to Female)	1.8	0.95-3.4	0.070

†Relative to ≥ 95% Adherence

Table 4.6. Factors Associated with Virologic Suppression with Allowance for Different Adherence-Suppression Relationships in Maintenance and Induction Therapy

Factor	Type of Therapy	Odds Ratio	95% Confidence Interval	p-value
Adherence 90-95%†	Maintenance	0.75	0.20-2.8	0.66
	Induction	1.2	0.34-4.6	0.74
Adherence 80-90%†	Maintenance	0.18	0.05-0.68	0.011
	Induction	1.6	0.68-3.8	0.28
Adherence <80%†£	Maintenance	0.81	0.18-3.7	0.78
	Induction	1.1	0.34-3.6	0.87
Treatment Type (PI relative to NNRTI)	Maintenance and Induction	1.2	0.59-2.3	0.65
Baseline Age (per 10 year increase in age)	Maintenance and Induction	1.4	0.89-2.3	0.14
Sex (Male relative to Female)	Maintenance and Induction	1.9	0.98-3.63	0.058

†Relative to ≥ 95% Adherence.
£Due to high rates of virologic suppression and the small number of patients in the <70% category for maintenance therapy, it was necessary to collapse the <70% and 70-80% categories.

Lastly, in the 44 patients who achieved virologic suppression anytime during the study period, 23 (52.3%) and 21 (47.7%) had overall adherence ≥ 95% and < 95% respectively. In the ≥ 95% group, mean adherence across patients was 98.8%, whereas the < 95% group had mean adherence of 78.9%. In the high adherence group, 6/23 (26.1%) patients were observed to fail over a mean follow-up time of 221 days. In the low adherence group, 8/21 (38.1%) patients experienced virologic breakthrough over a mean follow-up time of 190 days. Nevertheless, no statistically significant differences between the times to virologic failure were detected (Figure 4.5, p-value=0.37).

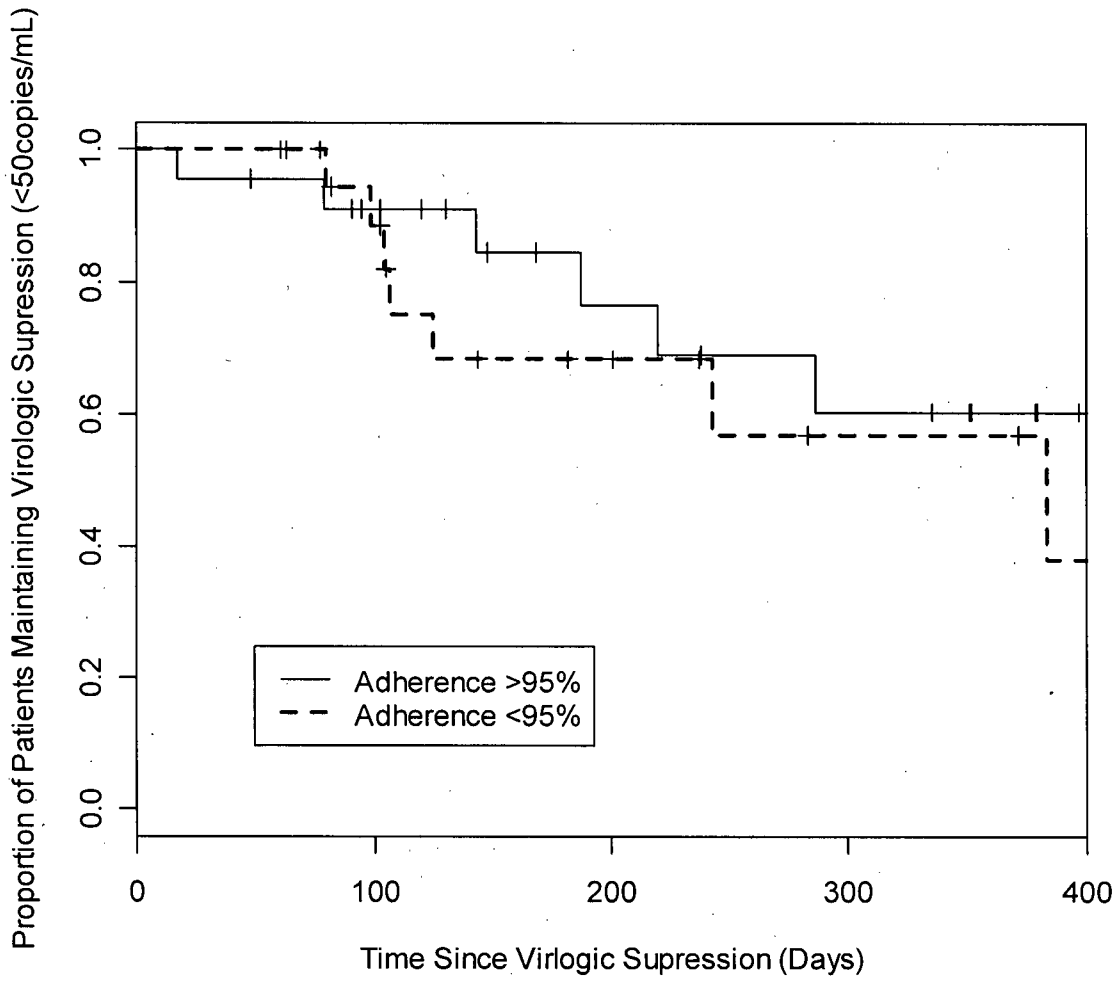

Figure 4.5. Kaplan-Meier curves for patients maintaining viral suppression (< 50 copies/mL)

4.4 Discussion

Our results indicate that the relationship between virologic failure and adherence may be much more subtle than previously reported. Excluding intervals with adherence between 80% and 90%, high rates of virologic suppression 92/105 (87.6%) were observed in intervals of maintenance therapy. Of these 13 failure intervals, only 2/105 (1.9%) resulted in a viral load

> 400 copies/mL. The much higher rate of virologic failure in the 80-90% adherence category during maintenance therapy (Figure 4.3 and Table 4.6) may indicate the existence of an intermediate threshold for the emergence of drug resistance. It should be noted however, that the description of this subgroup is a result of multiple comparison, and we are not able to exclude the possibility that this finding is a result of performing multiple tests on the initial model's three two-term interactions. On the other hand, the concept of an intermediate threshold has been previously reported [9-11, 52], and our findings are consistent with this notion. With increased potency and more favourable pharmacokinetic profiles, the current NNRTI- and boosted PI-based regimens may be more forgiving to periods of non-adherence than combinations evaluated in previous studies, such as those of Paterson [5] (primarily protease inhibitors without ritonavir-boosting). Despite the use of repeated measurements on each patient and having a sample size that is comparable to that of previous studies [5, 9, 44, 45], we were not able to detect a statistically significant effect of adherence on the probability of virologic suppression (Table 4.4 and Table 4.5). Hence, our data suggest that the previously reported high adherence thresholds ($\geq$ 95%) required for all HAART regimens under all circumstances may no longer be valid. In particular, when the multiple logistic regression analysis reported in Table 4.5 was simplified to compare the rates of virologic suppression in intervals with $\geq$ 95% adherence versus those with adherence < 95%, adherence $\geq$ 95% was not predictive of virologic suppression (OR = 1.2, 95% CI: 0.61-2.3, p-value=0.62).

Others have reported differing relationships between adherence and virologic suppression in NNRTI- and PI-based HAART [46]. We were unable to detect such differences in this study, possibly due to the roughly 3:1 imbalance in the number of intervals contributed by NNRTI- and PI-based HAART in our cohort.

Using methadone as a surrogate for HAART adherence is a useful alternative to previously used methods of measuring adherence. This method has benefits over the current gold-standard of electronic medication monitoring as it does not suffer from so-called "pocket-doses" or "curiosity-events" where pill bottles are opened for reasons other than administering the drug [4]. Moreover, this method provides a reasonable mechanism to ensure that all the components of the HAART regimen are taken, whereas electronic monitoring is usually only done selectively on one of the components.

Although significant efforts were made to ensure the surrogacy assumption was suitable, it is plausible that in certain patients surrogacy may not be appropriate (i.e. the patient consumed methadone but not HAART). Therefore, estimates of HAART adherence based on methadone adherence should be considered upper bounds for HAART adherence. It is hypothesized that problems with surrogacy would be more severe in the intervals of induction therapy compared to maintenance therapy. It seems reasonable to assume surrogacy in patients undergoing maintenance therapy, as initial viral suppression would have occurred only if drugs were being taken. However, in induction therapy, this argument cannot be applied.

If surrogacy in the intervals during induction therapy is questionable, it is possible that this is diluting any effect of adherence and that probability of virologic suppression increases more rapidly during induction therapy as adherence increases. This hypothesis could be tested using an alternative well-characterized method of measuring adherence that avoids the issue of surrogacy. However, in the maintenance therapy case, any dilution of the effect of adherence is

likely minor and it would be expected that the robust response across multiple levels of adherence would hold even in the case of perfect surrogacy.

It might be argued that our study does not have sufficient power to evaluate the effect of adherence on virologic suppression. The fact that we are suggesting that virologic suppression can occur and be maintained across a broad range of adherence thresholds might be viewed as suggesting that our sample size is insufficient to detect any true biologic differences that may be present. We feel this conclusion is incorrect for at least three important reasons. First, however we analyze our findings, no gradient is observed even for extremes of adherence (i.e. comparing "very low" to "very high" thresholds). If differences were present, we would expect to see such a gradient developing, even if perhaps not quite achieving statistical significance. Second, we are able to describe a sub-population with 80-90% adherence who appear to be experiencing virologic failure more frequently. These may well represent a group in whom the pattern of non-adherence is unique and more associated with a lack of long-term treatment efficacy. The identification of such a sub-population suggests that we are able to detect even subtle interactions between adherence and efficacy, if present. Finally, in comparison to the landmark publication of Paterson et al [5], we would be clearly able to detect such dramatic differences in efficacy if they were present. For instance, in Figure 4.4, the maximal width of the confidence bands is approximately 16%. Paterson et al observed a dramatic difference of nearly 60% between the highest adherence group (> 95%) and the lowest adherence group (< 70%). Obviously we would be able to detect differences of this magnitude, and our inability to do so suggests that the model presented in their publication may be constrained by the types of medication available at the time their study was completed and may no longer apply today.

Many have argued that IDUs would be unable to adhere to complex HAART regimens and therefore treatment should be withheld from them until they are more able to do so. This study has demonstrated that with DOT, IDUs achieved an average adherence rate of 90%, with 40% of patients having adherence >95%. When taken together with the results obtained in other cohorts [5-7, 44, 45], it is apparent that IDUs in our DOT program can adhere to HAART as well as, or more effectively than, non-IDUs in clinical practice. Further, it may be that the levels of adherence that are required for virologic efficacy have been overestimated and need to be re-evaluated in this era of more potent and simpler regimens. Finally, it is possible that "moderate (10-20%)" non-adherence carries with it an unacceptably high risk of failure. A better understanding of this phenomenon may allow us to select regimens with a higher threshold to resistance in patients thought to be at risk of this pattern of non-adherence to mitigate the consequences of failure to maintain virologic suppression.

5 Intermediate Adherence Thresholds to PI and NNRTI-based HAART and Empirical Models for the Development of Drug Resistance Mutations

5.1 Background

Adherence to HAART has been repeatedly shown to be a strong predictor of success for HIV-related therapeutic outcomes, including viral suppression [5-7], CD4+ cell increase [12, 13], antiretroviral drug resistance [6, 9, 53], progression to AIDS [14], and mortality [15]. Despite the apparent abundance of data on these topics, the quantitative relationship between adherence and these outcomes is still poorly understood, especially in HAART regimens reflecting the current standard of care. In fact, recent reports suggest that the previously described requirement of >95% adherence required for viral suppression may not be applicable in all circumstances to all patients who use NNRTI- or boosted PI-based HAART regimens [46, 47, 54].

The relationship between non-adherence and the risk of accumulation of drug resistance mutations (DRMs) has been hypothesized to follow a 'bell-shaped' curve [55]. Adherence below a certain level is thought not to supply enough of a selective advantage to drug resistant mutants. On the other hand, high adherence provides a consistently high level of drug concentration thought to inhibit replication so strongly that the number of viral replication events is too small for mutant viruses to emerge. Several reports have provided evidence in support of this hypothesis and suggested that such a bell-shaped curve does exist for PI-based regimens [10, 11, 52]. Data verifying this relationship for NNRTIs have thus far not been reported.

Studies assessing this relationship have typically taken a cross-sectional approach [11, 52], relating the average adherence over some long period of time (typically >6 months) to some distant therapeutic outcome. Such study designs are limited in their ability to detect the effects of periodic lapses in adherence. Furthermore, isolates with some mutations that would eventually result in high-level genotypic resistance if replication were allowed to continue in the presence of selective drug pressure would largely go undetected, as the primary outcome is based on the last genotypic resistance test available. For this and other reasons, we and others [48] advocate using longitudinal approaches to study the complex interaction between adherence and resistance. With this in mind, we have evaluated this relationship in a cohort of IDUs receiving contemporary HAART regimens within a DOT program.

5.2 Methods

5.2.1 Study Design and Treatment Program

This retrospective longitudinal study was conducted at the Pender Community Health Center, a multidisciplinary clinic located in the Downtown Eastside of Vancouver. Patients were eligible for inclusion in this study if they were HIV-infected and enrolled in the clinic's DOT HAART administration program as well as the methadone maintenance program during the study period, October 2002 to May 2006. Within these programs, HIV plasma viral load tests are performed as the standard of care, approximately every three months or as clinically indicated. HIV plasma viral load was measured using the Amplicor HIV-1 Monitor assay (Roche Diagnostics, Mississauga, ON) and CD4+ cell counts were measured by flow cytometry at a local certified reference laboratory. Genotypic resistance testing is routinely done in patients who have

HIV plasma viral load >250 copies/mL using the genotypic results obtained from the VirtualPhenotype assay (VIRCO Labs, Mechelen, Belgium). Additionally, plasma samples are frozen and available for retrospective testing in cases where genotypic testing has not been previously ordered.

Patients enrolled in both the DOT and methadone maintenance programs present daily to a community pharmacist, where both HAART and methadone are administered onsite [17, 18]. Hence, in patients who receive once-daily HAART regimens, daily methadone pharmacy dispensing records can be used as a surrogate for administration of HAART.

5.2.2 Data Collection

All intervals of the time between two successive viral load measurements (usually approximately 3 months) were identified for each eligible patient. Time intervals included in this study were restricted to those where patients were prescribed HAART as indicated by chart notes and prescription records. Intervals where treatment was discontinued prior to the end of the interval (i.e. before the subsequent viral load measurement) or where methadone adherence data was unavailable were omitted from analysis. However, in cases where a patient had both eligible and ineligible intervals, all eligible intervals were included in the analysis. Due to the genotypic resistance assay sensitivity limitation, only intervals resulting in an end of interval HIV plasma viral load exceeding 250 copies/mL were eligible for analysis.

Baseline data including sex, age, antiretroviral (ARV) treatment history, CD4+ cell count, and HIV plasma viral load were recorded at the beginning of each patient's first eligible interval.

For each eligible interval, the components of the HAART regimen, HIV plasma viral load at the beginning and end of the interval, and methadone adherence (defined as the fraction of methadone doses consumed during the interval) were determined. Genotypic resistance testing was obtained for the oldest available plasma sample to detect horizontally transmitted genotypic drug resistance, as well as for any additional viral load measurements where the patient was receiving ARV therapy and had viral load exceeding 250 copies/mL. The cumulative genotypic mutation history of each patient up until and including the plasma sample obtained at the beginning of the interval was used to estimate genotypic mutations likely present. Based on this definition, a mutation is considered to be emergent only if it had not been detected in isolates previously, or at, the beginning of the interval. Intervals beginning with HIV plasma viral loads below the VirtualPhenotype assay's limitations (sensitive in samples with viral load > 250 copies/mL) were classified as having developed no new mutations since the most recent previous genotypic test. Genotypic drug resistance was identified based on the IAS-USA resistance table (November, 2005 revision) [56].

5.2.3 Statistical Analysis

The impact of adherence on the accumulation of genotypic resistance mutations was examined longitudinally. Poisson regression models were fit using the number (count) of new DRMs detected in the end of interval plasma sample. An offset term was included in all such models to account for the variable interval lengths; estimates derived from such models are then appropriately standardized to a unit time (as opposed to unit interval). Additionally, to account for the correlated nature of the repeated measurements on each patient, the generalized estimating

equation (GEE) method [22] was used with an independence working correlation structure to fit the Poisson regression models.

Estimated HAART adherence, treatment type (NNRTI- or PI-based HAART), virologic status at the most recent previous viral load measure (i.e. detectable or undetectable), and HIV plasma viral load at the beginning and end of each eligible interval were considered as predictors of the rate of accumulation of new DRMs. Adherence was categorized in two ways. The first approach categorizes it into the three levels < 80%, 80-90%, and > 90%, while the second categorizes it as being between 80% and 90% or not. Both these approaches focus on the 80%-90% adherence interval previously suggested to be important for the development of drug resistance [10, 11, 52], with the former approach allowing one to evaluate differences that may exist in the rate of accumulation of DRMs above and below this threshold. Beginning of interval HIV plasma viral load was categorized as < 50, 50-1000, 1000-10000, and > 10000 copies/mL, while end of interval HIV plasma viral load was categorized as < 1000, 1000-10000, and > 10000 copies/mL. Sex and age at entry to the study as well as time since entry into the study were investigated as additional predictors.

Models were first fit separately for each predictor by univariate analyses, for the total, PI-associated and RT-associated number of new DRMs. When evaluating PI-associated mutations in this and subsequent analyses, the analysis was restricted to those taking PI-based HAART as patients not receiving PI-based HAART have no selective pressure allowing for emergence of these resistant isolates.

To allow for greater flexibility in fitting separate models relating adherence and HIV plasma viral loads (both as continuous variables) to rates of accumulation of new DRMs, natural cubic splines were used in Poisson regression models (using the 'ns' implementation within R version 2.3.1 [57]). This approach allows for the development of empirical models describing relationships that may be non-linear. Knots were initially placed by a qualitative assessment of a scatterplot of the data, placing knots at levels of adherence where the greatest amount of variation was observed. Both the location and number of knots were subsequently evaluated by a sensitivity analysis. The final choice of spline fit was guided by the objective of using as few knots as possible (parsimony), while still adhering to the main pattern of the apparent relationship (goodness-of-fit).

Lastly, multiple Poisson regression models were fit for each of total, PI- and RT-associated DRMs. Guided by the results of the univariate analyses, the following predictors were included in the initial model: adherence (80-90% vs. > 90% or < 80%), treatment type (NNRTI- vs. PI-based HAART), baseline age, sex, log (base 10) of the measurement of end of interval viral load, and viral suppression at the beginning of the interval. The model was then simplified using backwards elimination, with the adherence predictor retained in the model throughout. All reported p-values are two-sided; p-values less than 0.05 were considered statistically significant.

5.3 Results

Of the 77 patients eligible for this study, 45 had at least one HIV plasma viral load exceeding 250 copies/mL allowing for generation of genotypic resistance assay results. Of these eligible 45 patients, 36 contributed at least one interval to the analysis, with three being excluded

because all intervals under observation occurred while they were taking at least one component of their HAART regimen twice or three times a day, which does not allow for direct measurement of HAART adherence by methadone adherence surrogacy. The remaining six patients were excluded because of unavailability of historic plasma samples to evaluate genotypic resistance at either the beginning or end of all of their intervals.

The baseline and clinical characteristics of these eligible patients are presented in Table 5.1. These 36 eligible patients contributed a total of 85 time intervals, with a median number of intervals per patient of 2.0 (range 1-5). The mean interval length spanned 80 days, which corresponds with the current interval for monitoring HIV plasma viral load, and the patients were followed for a median of 183 days. Characteristics of the 85 time intervals are presented in Table 5.2.

Table 5.1. Patient Characteristics

N	36
Female	11 (30.6%)
Male	25 (69.4%)
Mean Follow-up (days, SD)	188 (123)
Median Number of Intervals Contributed (range)	2.0 (1-5)
Mean Baseline Age (years, SD)	38.5 (7.1)
Median Baseline HIV Plasma Viral Load (copies/mL, IQR)	9925 (86461)
Mean Baseline CD4+ Cell Count (cells/mm^3, SD)	191 (140)
Mean Adherence for all Follow-up (SD)	85.0% (14.0%)
ARV Naïve at Baseline	3 (8.3%)
Median Baseline Drug Experience (# of NNRTIs/ # of PIs / # NRTIs)	1/1/3.5

Table 5.2. Interval Characteristics

N	85
Therapy Type	
NNRTI-based Therapy	17 (20.0%)
PI-based Therapy	68 (80.0%)
Virologic Status at the Beginning of the Interval	
Full Virologic Suppression (<50 copies/mL)	12 (14.1%)
Detectable HIV Plasma Viral Load (>50 copies/mL)	73 (85.9%)
Median HIV Plasma Viral Load at Beginning of Interval (copies/mL, IQR)	3100 (68814)
Median HIV Plasma Viral Load at the End of the Interval (copies/mL, IQR)	3420 (42229)
Mean Interval Length (days, SD)	80.0 (48.4)
Mean Interval Adherence (SD)	87.3% (15.1%)

Mean patient adherence over the study period was 85.0% (SD: 14.0%), with 10 (27.8%) and 26 (72.2%) of patients exhibiting > 95% and > 80% adherence respectively. Only 18 (21.2%) of the 85 intervals resulted in new DRMs, with 4 NNRTI-, 9 NRTI-, and 15 PI-associated DRMs being detected.

As presented in Table 5.3, intervals where adherence was between 80% and 90% resulted in higher unadjusted rates of DRMs (2.8 DRMs/year) than intervals with < 80% adherence (1.1 DRMs/year) and > 90% adherence (1.5 DRMs/year). In PI-based HAART, total mutations were accumulated at a rate of 1.08 DRMs/year (SD=3.4), with 0.8 DRMs/year (SD=3.0) and 0.3 DRMs/year (SD=1.7) being PI- and NRTI-associated mutations respectively. We also identified 8 intervals during which patients were receiving unboosted PIs (atazanavir in all cases). In these 8 intervals, no new PI- or NRTI-associated DRMs were observed. In NNRTI-based HAART, total mutations were accumulated at a rate of 3.8 DRMs/year (SD=7.3), with 1.5 DRMs/year (SD=3.7) and 2.3 DRMs/year (SD=4.4) being NNRTI- and NRTI-associated mutations respectively.

Table 5.3. Mean Rates of Drug Resistance Mutations Stratified by Level of Adherence

	Overall (n=85)	< 80% Adherence (n=20)	80-90% Adherence (n=20)	> 90% Adherence (n=45)
Rate of Accumulation of New DRMs, per year (SD)	1.8 (4.6)	1.1 (2.8)	2.8 (5.5)	1.5 (4.7)
Rate of Accumulation of New Protease Inhibitor DRMs, per year* (SD)	0.8 (3.0)	0 (N/A)	2.0 (5.3)	0.5 (1.9)
Rate of Accumulation of New Reverse Transcriptase DRMs, per year (SD)	1.0 (3.8)	1.1 (2.8)	0.9 (3.0)	1.0 (4.5)

*Restricted to intervals where PIs were being used (n=68, 15, 17, and 36 respectively)

The rates of accumulation of DRMs stratified by beginning and end of interval HIV plasma viral load levels are presented in Tables 5.4 and 5.5 respectively. DRMs were generally accumulated at higher rates as the end of interval viral load increased. However, no trend was apparent in the rates of DRM accumulation as a function of the beginning of interval viral load.

Table 5.4. Mean Rates of Drug Resistance Mutations Stratified by Plasma Viral Load in Sample at Beginning of Interval

	< 50 copies/mL (n=12)	50-1000 copies/mL (n=17)	1000-10000 copies/mL (n=22)	>10000 copies/mL (n=34)
Rate of Accumulation of New DRMs, per year (SD)	1.6 (3.2)	0.9 (1.9)	2.6 (6.5)	1.6 (4.4)
Rate of Accumulation of New Protease Inhibitor DRMs, per year* (SD)	1.3 (3.7)	0.5 (1.5)	0.9 (2.4)	0.7 (3.8)
Rate of Accumulation of New Reverse Transcriptase DRMs, per year (SD)	0.4 (1.2)	0.4 (1.3)	1.8 (6.3)	1.0 (3.0)

*Restricted to intervals where PIs were being used (n= 8, 16, 17 and 27 respectively)

Table 5.5. Mean Rates of Drug Resistance Mutations Stratified by Plasma Viral Load in Sample Used for Genotyping

	< 400 copies/mL (n=4)	400-1000 copies/mL (n=18)	1000-10000 copies/mL (n=30)	>10000 copies/mL (n=33)
Rate of Accumulation of New DRMs, per year (SD)	0 (N/A)	1.1 (2.7)	1.6 (3.1)	2.4 (6.3)
Rate of Accumulation of New Protease Inhibitor DRMs, per year* (SD)	0 (N/A)	1.1 (2.8)	0.4 (1.8)	1.1 (4.2)
Rate of Accumulation of New Reverse Transcriptase DRMs, per year (SD)	0 (N/A)	0 (N/A)	1.0 (2.8)	1.7 (5.5)

*Restricted to intervals where PIs were being used (n= 4, 17, 23 and 24 respectively)

Exploration of the correlation structure of the logarithm of the rates of accumulation within individual patients indicated that the correlations were modest ($|r|<0.3$), with no clear pattern relating to the time separation. There are biological arguments to suggest that in certain contexts the correlation between any two observations could be positive or negative. Furthermore, our definition of new DRMs precludes us from counting the same DRM (if present) at a subsequent interval, and therefore one may expect that the correlation between any two time points to be negative as DRMs are accumulated over time. Given the absence of a clear indication of a pattern, we proceeded with an independence working correlation structure to fit the Poisson regression models with GEE.

The results of the univariate Poisson regression analyses are provided in Table 5.6. PI-associated DRMs were accumulated at a much higher rate in intervals where adherence was between 80% and 90% (Relative Rate (RR) = 6.82, 95% CI: 1.38-33.76, p-value=0.019), however adherence was not predictive of higher rates of total or RT-associated DRMs. Intervals

where patients received PI-based HAART were associated with significantly less risk of accumulating total DRMs (RR = 0.30, 95% CI: 0.10-0.89, p-value=0.030) and RT-associated DRMs (RR = 0.08, 95% CI: 0.02-0.32, p-value<0.001). The viral load at the end of the interval may be predictive of the number of new PI-associated DRMs (p-value=0.074), suggesting that this relationship should be explored further. Furthermore, this indicates that one may want to consider adjusting for its effect in subsequent analyses.

Table 5.6. Univariate Poisson Regression for Rate of Accumulation of Drug Resistance Mutations

Factor	Total DRMs Relative Rate (95% CI)	p-value	PI DRMs Relative Rate (95% CI)	p-value	RT DRMs Relative Rate (95% CI)	p-value
PI-based HAART	0.30 (0.10-0.89)	0.030	N/A	N/A	0.08 (0.02-0.32)	<0.001
Adherence (relative to >90%)						
80-90%	1.93 (0.64-5.83)	0.24	3.97 (0.83-19.07)**	0.085	0.50 (0.10-2.52)	0.40
<80%	0.81 (0.28-2.36)	0.70	Excluded**		1.35 (0.36-5.10)	0.66
80-90% Adherence	2.09 (0.74-5.89)	0.16	6.82 (1.38-33.76)	0.019	0.44 (0.10-1.88)	0.27
Age (per 10 year Increase)	0.67 (0.34-1.30)	0.23	0.32 (0.08-1.32)	0.12	0.88 (0.38-2.07)	0.77
Sex (Male relative to Female)	1.02 (0.29-3.64)	0.97	0.80 (0.10-6.31)	0.83	1.37 (0.24-7.77)	0.73
Suppressed Viral Load at Beginning of Interval	1.09 (0.31-3.88)	0.89	1.96 (0.26-14.62)	0.51	0.33 (0.06-2.02)	0.23
Beginning of Interval Viral Load (relative to <50 copies/mL)						
50-1000 copies/mL	0.58 (0.17-1.93)	0.37	0.36 (0.08-1.64)	0.19	1.74 (0.21-14.25)	0.61
1000-10000 copies/mL	1.06 (0.26-4.25)	0.93	0.43 (0.05-3.74)	0.45	3.64 (0.48-27.73)	0.21
>10000 copies/mL	1.04 (0.23-4.80)	0.96	0.67 (0.04-9.65)	0.77	3.40 (0.48-24.03)	0.22
End of Interval Viral Load (relative to <1000 copies/mL)						
1000-10000 copies/mL	1.00 (0.22-4.59)	0.99	0.12 (0.01-1.30)	0.082	Excluded***	
>10000 copies/mL	1.69 (0.32-8.89)	0.54	0.80 (0.10-6.63)	0.84	2.71 (0.79-9.31)***	0.11

** Zero PI mutations occurred for <80% adherence, hence this category was excluded from this assessment, and 80-90% adherence was compared relative to only >90% adherence

*** Zero RT mutations occurred for end of interval viral loads <1000 copies/mL, so this assessment compares ≤10,000 copies/mL to >10,000 copies/mL

Motivated by the non-linear patterns suggested by the univariate analyses, the relationship between adherence and the rate of accumulation of new DRMs was then explored using natural

cubic splines with adherence as a continuous predictor. The sensitivity analysis entailed placing a limited number (two or three) of interior spline knots at different locations on the adherence scale and increasing or decreasing the number of knots; in nearly every case, a 'bell shaped' curve resulted but adding too many knots resulted in unusual patterns. For example, when additional knots were placed at levels of adherence > 90%, a minimum was observed around 95% adherence, followed by a rapid increase in the fitted rate of accumulation from 95% to 100% adherence. Such a pattern is likely an artefact as such a relationship is very unlikely. Given our knowledge about this relationship, a simple concave relationship seemed most plausible, and we proceeded to fit models with the fewest degrees a freedom that consistently produced this kind of relationship (two interior knots).

The final fitted forms of these relationships, based on two interior knots placed at 70% and 80% adherence, are shown in Figure 5.1. (Due to limitations in the data set, the two interior knots instead were placed at 60% and 70% adherence for the curve fit for total DRMs during NNRTI-based HAART.) Total new DRMs in intervals with subjects receiving NNRTI- or PI-based HAART were estimated to occur at a maximum rate of 2.1 DRMs/year at about 85% adherence and decreased when adherence increased to 100% (1.3 DRM/year). During NNRTI-based HAART, the maximum total accumulation was estimated at 4.5 DRMs/year at about 70% adherence, and accumulation at 100% adherence was estimated at 2.5 DRMs/year. Restricting the analysis to PI-based HAART, maximum rates of accumulation were estimated to occur at about 87% (1.8 DRMs/year) and 90% (2.7 DRMs/year) adherence for total and PI-associated DRMs respectively.

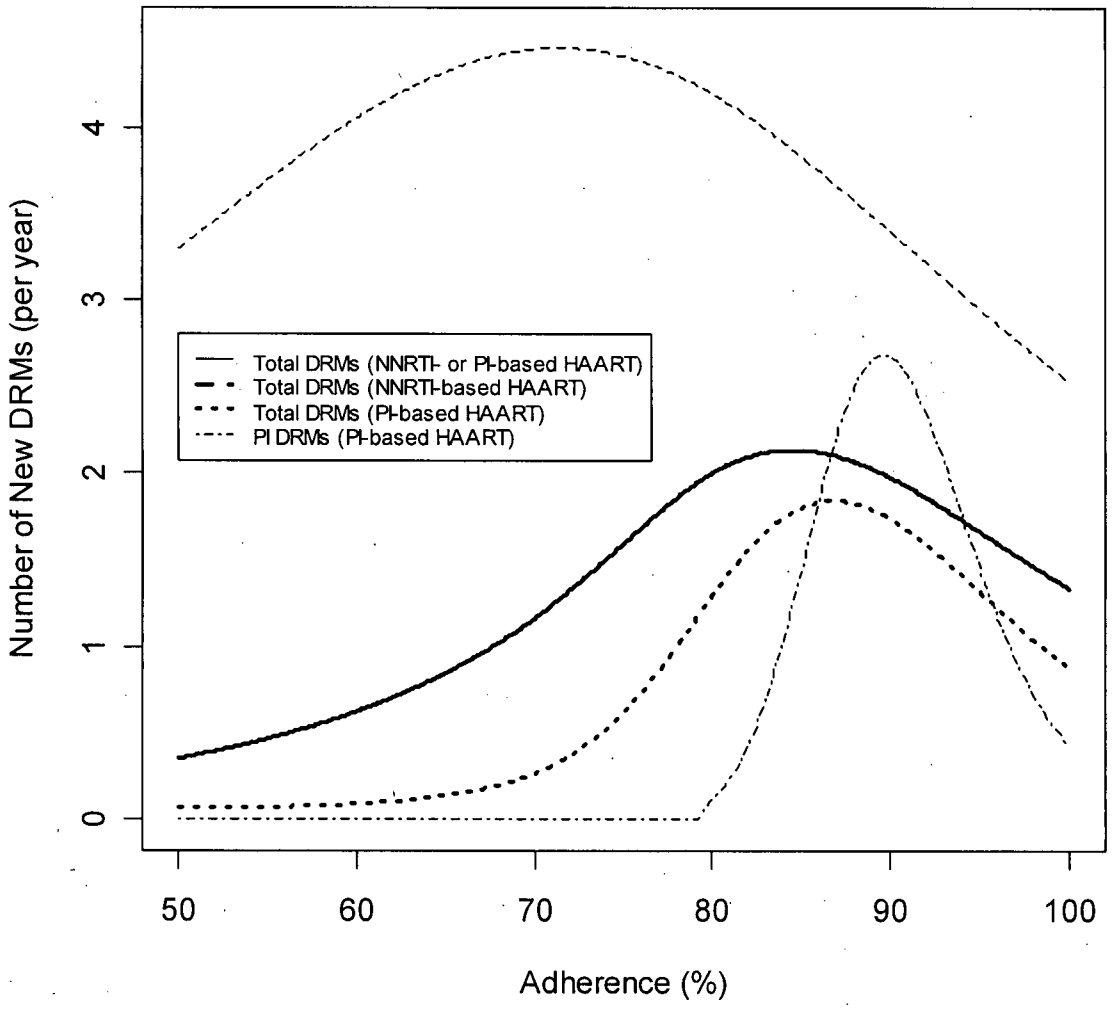

Figure 5.1. Relationship between HAART adherence and the rate of accumulation of drug resistance mutations (DRMs)

To gauge the precision of the curves presented in Figure 5.1, the same models were fit to each of 200 bootstrap samples. Figure 5.2 presents pointwise 90% confidence bands based on the spline fits for the 200 bootstrap samples, together with the original spline fit and the raw data, for the rate of accumulation of total DRMs in these 36 patients. Figure 5.3 presents the same plot

for new PI-associated DRMs in the 30 patients with intervals of PI-based HAART. Due to the limited amount of data available, these approximate confidence bands are quite wide. The limited number of non-zero DRM counts would create some bootstrap samples consisting of nearly all zero counts. In this case, the bootstrapped relationship would estimate a rate of new DRMs of nearly zero across all levels of adherence. Despite the limited amount of data, the 90% confidence bands clearly suggest a concave ('bell shaped') curve.

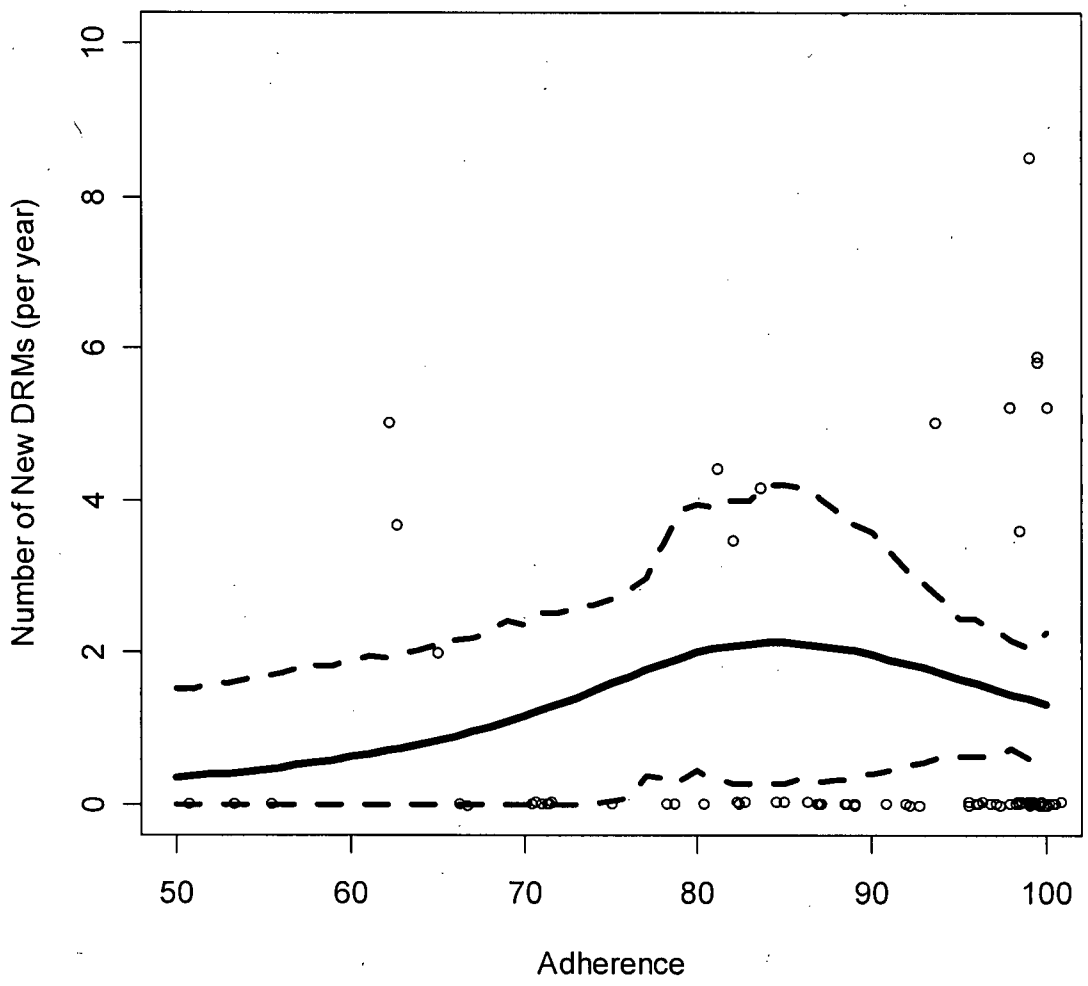

Figure 5.2. Relationship between HAART adherence and the rate of accumulation of total DRMs, together with the pointwise 5th and 95th percentiles of the estimated curves obtained from 200 bootstrap samples

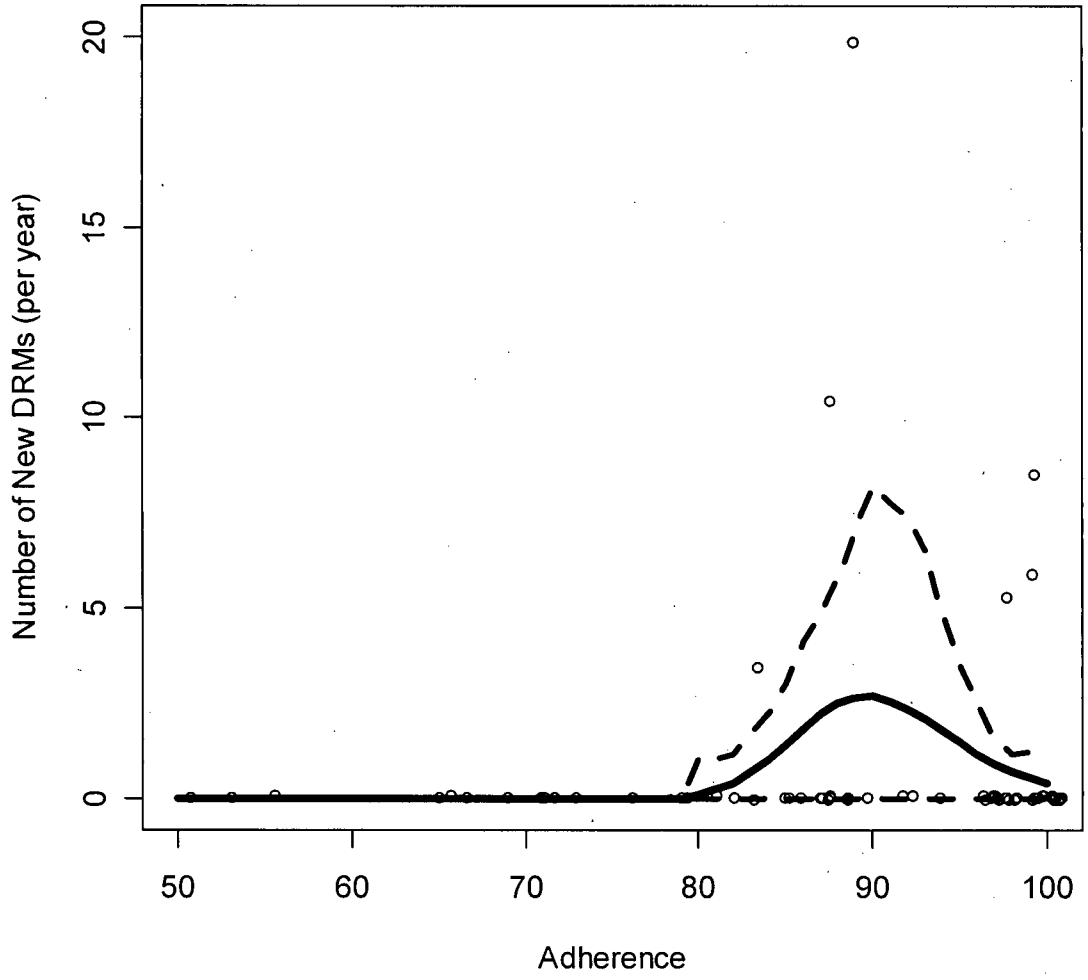

Figure 5.3. Relationship between HAART adherence and the rate of accumulation of PI-associated DRMs, together with the pointwise 5th and 95th percentiles of the estimated curves obtained from 200 bootstrap samples

The univariate analyses conducted above suggested that end of interval viral load (as a categorical predictor) may be important in predicting the rate of accumulation of PI-associated DRMs. With this in mind, the relationship between the rate of accumulation of new PI-

associated DRMs and the logarithm (base 10) of the end of interval viral load was similarly explored using natural cubic splines. The final fitted model had interior knots placed at end of interval viral loads of 250, 1,000 and 10,000 copies/mL and estimates a maximum rate of 4.4 DRMs/year at a HIV plasma viral load of about 690 copies/mL (Figure 5.4). After this peak, the fit predicts a rapid decrease in rates of DRM accumulation until a minimum of essentially 0 DRMs/year at HIV plasma viral load of about 3090 copies/mL, followed by a monotonic increase in the rate of accumulation of DRMs. The sensitivity analysis that guided the choice of final form of this fit indicated that moving all the interior knots to the right of the maximum observed at a HIV plasma viral of 690 copies/mL would yield a 'U-shaped' curve, an unlikely form for this relationship. Similarly, including additional interior knots produced oscillations to the monotonic increase observed from 3,090 to 100,000 copies/mL, contrary to biological expectation. Approximate 90% confidence bands were estimated from bootstrap samples as with adherence. The resulting bands mirror the estimated relationship quite closely, but suggest two additional maxima. This is not surprising as these maxima occur at the only other end of interval plasma viral loads where PI-associated DRMs were observed to occur.

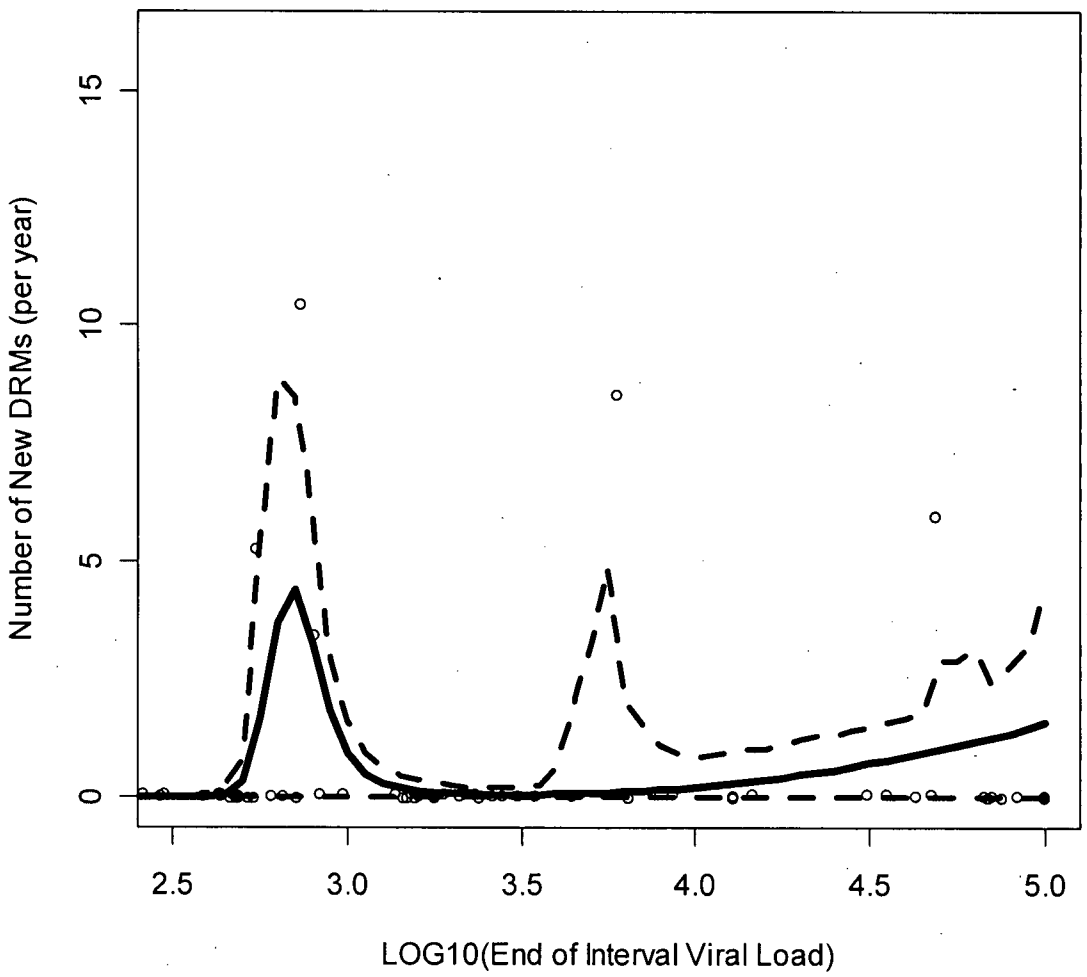

Figure 5.4. Rate of new PI drug resistance mutations (DRMs) as a function of the logarithm (base 10) of the end of interval HIV plasma viral load together with the pointwise 5th and 95th percentiles of the estimated curves obtained from 200 bootstrap samples

In multiple Poisson regression models adjusting for the end of interval HIV plasma viral load using natural cubic splines of the form suggested by the exploratory work described above (due to the limited number of RT mutations occurring at viral loads < 1,000 copies/mL, a different form of spline was used, placing knots instead at 1,000 and 10,000 copies/mL), after

model reduction using backwards elimination on the other predictors included in the initial model, PI-based HAART was associated with clear and substantial reductions in the rate of new total (RR = 0.25, 95% CI: 0.11-0.62, p-value=0.002) and NRTI-associated DRMs (RR = 0.12, 95% CI: 0.04-0.44, p-value=0.001). In the same analyses, interval adherence between 80% and 90% was associated with high rates of total (RR = 3.24, 95% CI: 1.04-16.05, p-value=0.042) and PI-associated DRMs (RR = 4.42, 95%CI: 1.26-15.48, p-value=0.020) in intervals using PI-based HAART. However, interval adherence between 80% and 90% was not predictive of total (RR = 1.79 95% CI: 0.70-4.60, p-value=0.23) nor NRTI-associated DRMs (RR = 0.50, 95% CI: 0.14-1.75, p-value=0.28). Qualitatively similar results were obtained when end of interval viral load was instead incorporated into the model as a categorical variable (with categories ≤ 10,000 and > 10,000 copies/mL for the RT DRM analysis as in Table 5.6).

Table 5.7. Multiple Poisson Regression for Rate of Accumulation of Drug Resistance Mutations

Factor	Total DRMs Relative Rate (95% CI)*	p-value	Total DRMs** Relative Rate (95% CI)*	p-value	PI DRMs Relative Rate (95% CI)*	p-value	RT DRMs Relative Rate (95% CI)*	p-value
PI-based HAART	0.25 (0.11-0.62)	0.002	N/A	N/A	N/A	N/A	0.12 (0.04-0.44)	0.001
80-90% Adherence	1.79 (0.70-4.60)	0.23	3.24 (1.04-10.05)	0.042	4.42 (1.26-15.48)	0.020	0.50 (0.14-1.75)	0.28

*Adjusted for end of interval HIV plasma viral load as a continuous variable, using natural cubic splines
** In PI-based HAART

5.4 Discussion

These data suggest that the relationship between the rate of accumulation of new DRMs and adherence can be described as a 'bell-shaped' curve, which is consistent with other reports on

this topic [11, 52]. With PI-based HAART, both total and PI-associated DRMs appear to be accumulated at a maximum rate between 80% and 90% adherence. However, when NNRTIs are used, rates of accumulation are consistently higher and maximal rates of DRM accumulation are observed at lower rates of adherence.

These findings are consistent with other reports describing the shape of the resistance-adherence curve, but our approach to the analysis offers the ability to better appreciate additional characteristics of the curve. Bangsberg and colleagues report empirically derived curves modelled for PI-based HAART assuming the independence of virologic and resistance outcomes [52]. The resulting curve is much more diffuse than that presented here, with non-nominal rates of resistance (i.e. >0.5 total DRMs/year) occurring even at levels of adherence <30%. In this study, in intervals of PI-based HAART, not a single PI-associated DRM and only 3 NRTI-associated DRMs were observed when adherence was less than 80%. Our resulting curve is much narrower than that reported by Bangsberg et al., but agrees in the level of adherence associated with the maximum likelihood of accumulation of total DRMs, occurring at about 87% in both studies.

Similarly, King and colleagues model this relationship directly using local linear models and polynomial regression [11]. Their resulting PI-associated DRM curves are much narrower than those reported by Bangsberg et al., but still much wider than those presented in this paper. We believe this is due to the fact that very different study designs were used in the two previous reports, in which adherence is averaged over the 6-month study period and genotypic resistance is only evaluated at the beginning and end of the study period. This method could suffer from dilution of the actual effect of non-adherence because patients may be misclassified if they have

substantial variability in their adherence patterns. In our study, any variability in adherence can be captured much more effectively because genotypic testing is collected whenever a patient is failing treatment and the results of these tests can be localized to the adherence level most proximate to it. If DRMs can result from short-term episodes of non-adherence, then long-term average adherence may be a poor instrument for assessing the relationship between adherence and DRMs. In the case of PI-associated DRMs, this may be indeed what we are observing as the range of adherence levels at which we observe DRMs is limited to adherence > 80%.

The finding that NNRTI-based therapy is strongly associated with up to a 4-fold increase in the rate of DRM accumulation is consistent with the known lower barrier of this class to drug resistance [58]. However, the relatively small number of intervals we were able to study may limit our conclusions. Additionally, despite having 39 (46%) intervals with lamivudine containing regimens, only 2 intervals resulted in a lamivudine associated DRM (M184V/I). Both occurred at relatively high rates of adherence (>90%), which is consistent for a drug with such a low barrier to resistance [59]. This supports the notion that even in ideal circumstances, near perfect adherence is needed (especially with a viral load that is not undetectable) to avoid the appearance of lamviudine associated DRMs [11].

The data relating the end of interval viral load to the accumulation of DRMs suggests that there may be two processes by which HIV accumulates DRMs. A mode may exist when the HIV plasma viral load is between 500 and 1000 copies/mL already selecting for the accumulation of most resistance mutations. The increase in the rate of DRMs that appears to occur when the end of interval viral load is greater than 3000 copies/mL may reflect the appearance of secondary PI

mutations, generated to correct the deficit in replication capacity caused by the genetic changes initially selected at lower HIV plasma viral load levels.

Although we are using a surrogate to measure HAART adherence, we believe it to be a useful and suitable alternative to other methods of measuring adherence. Restricting the analysis to patients receiving HAART once-daily essentially makes methadone adherence equivalent to daily observed ingestion of methadone by a community based pharmacist. With that said, it is possible that some patients may receive methadone, and not their HAART. We would expect that this problem would be more evident in patients with incomplete virologic suppression, as those with complete virologic suppression have taken at least some of their doses to achieve virologic suppression. Likewise, patients who accumulate DRMs would require the selective pressure obtained from taking drug. For patients not in either of these two categories, methadone should be considered an upper bound to estimate HAART adherence, and this may have the consequence of shifting the intermediate thresholds we are observing to lower levels of adherence. However, given that our data are consistent with previous reports regarding both the shape and the centering of the curve relating adherence to the rates of DRM accumulation [11, 52], we expect this phenomenon to have little or no effect on our data or conclusions.

Our data provide further support for the notion that the adherence thresholds previously defined for successful virologic suppression (i.e. > 95% adherence) may not be sufficient to prevent the accumulation of all DRMs. In fact, we estimate non-trivial rates of DRMs even at 100% adherence. Hence, strategies to improve adherence to HAART should be incorporated into routine clinical practice, especially shortly following its initiation while HIV plasma viral load is still high. Our data suggest that adherence levels contemporary to the breakthrough of the

resistant strain of HIV are important to understand the exact mechanism by which DRMs are being accumulated. In particular, in PI-based HAART, between 80% and 90% adherence, there may exist an environment predisposing to higher rates of DRMs than adherence above or below these thresholds. We hypothesize that the 80-90% threshold may represent a specific pattern of missing doses that leads to high rates of DRMs. Within these thresholds, patients may undergo periods of non-adherence which are relatively short and frequent in nature. This pattern of adherence may expose circulating virus to suboptimal drug concentrations, leading to the maximal selection of resistant isolates. Patterns of non-adherence when adherence exceeds 90% by its very nature must be infrequent, whereas patients with adherence < 80% can have longer but infrequent periods of non-adherence. If this hypothesis can be substantiated, it would drastically alter the way in which adherence to HAART is perceived and managed in clinical practice, at least until the HIV plasma viral load achieves undetectable levels.

6 Discussion

6.1 Key Findings

In Chapter 3, we presented a longitudinal study relating methadone adherence to illicit drug use and other important covariates. Typically studies assessing the impact of illicit drug use on methadone-related treatment outcomes such as abstinence and treatment retention have found that baseline or ongoing illicit drugs, in particular cocaine, have led to poorer outcomes. Our study, however, is the first on record to relate methadone adherence to ongoing illicit drug use. Often drug treatment program protocols contain provisions to discontinue methadone treatment if any illicit drugs are found to be present in urinalysis results. However, we have found that methadone maintenance can be a stabilizing force which can substantially improve outcomes for other diseases. Unfortunately, the literature and this thesis have both demonstrated that adherence is important for successful therapy of HIV-infection. Hence, any program that relies heavily on methadone to reinforce adherence to HAART needs to be aware of factors which may contribute to decreased rates of methadone adherence.

In Chapter 3, we present data suggesting that use of opiates, as would be expected, contributes to decreased rates of adherence. Conversely, higher methadone dose was associated with higher rates of methadone adherence. These two phenomena may be describing features of the methadone maintenance program at the Pender Community Health Center. A more complicated situation occurs when describing the effect of amphetamine use on methadone adherence. In patients taking amphetamines by themselves, our data suggest that they may

exhibit higher rates of methadone adherence. However, in patients taking both amphetamines and either cocaine or benzodiazepines, any positive effect associated with amphetamine use is negated.

In Chapter 4, we presented an analysis that suggests previously defined HAART adherence thresholds required for viral suppression may no longer be valid (at least in patients undergoing maintenance therapy). This suggests that alternative approaches to therapy which relax the strict requirements (> 95% adherence) presented in previous studies may be feasible. This is an area of research which is and should continue to be actively pursued. While patients were undergoing maintenance therapy, suppression was robust across adherence levels, with the exception of the 80%-90% adherence level where patients were observed to have a higher rate of virologic failure. This suggests that any alternative approach to therapy may want to attempt to avoid this adherence level. However, caution must be stressed when interpreting this finding, as it may be a result of performing multiple comparisons in the data analysis.

A possible reason for this observation was explored in Chapter 5, where we found higher rates of DRMs to occur during time intervals when PI-based HAART was being used at rates of adherence between 80% and 90%. This analysis also provides further evidence of the existence of a 'bell-curve' type relationship between adherence and the accumulation of DRMs.

6.2 Methodological Issues

While conducting the analysis presented in Chapter 3, we noted at least one other way the data could be structured. We have chosen to represent the data as a time interval consisting of the

time between two urinalysis results. However, since methadone administration is recorded on each day, another approach would be to use each day as an observation, with a binary response variable indicating whether or not a dose was ingested on that day. This approach then falls naturally into a logistic regression framework and has the additional benefit of being able to address day-varying covariates, such as day of the week. However, because the thresholds that have been established for both HIV and HCV therapy are often expressed in terms of percentage adherence over a time interval, we felt use of such a response to be more meaningful. The alternative approach, while being able to assess day-varying covariates, doesn't significantly improve measurement of our primary interest – illicit drugs; interpolation and extrapolation of the most proximate urinalysis results must be utilized in both approaches. Proceeding as we did with adherence as a percentage does leave open the possibility that we may predict in certain time intervals that methadone adherence may exceed 100%. This phenomenon was observed, but no interval was predicted to have more than 104% adherence, and > 100% adherence was only estimated a small minority of 24 intervals (4.9%).

In all chapters we were able to note specific benefits of using a longitudinal study design and corresponding statistical methodology over those traditionally used in such studies. For each of the three studies in this thesis, a cross sectional approach is typically used, in which the data for each patient across all time periods is summarized so that it can be analyzed using basic statistical methodology. This approach does not make efficient use all of the study data if clinical endpoints are measured at several time points. With this in mind, the studies presented here have more statistical power than those presented in the literature with similar sized study populations.

Using longitudinal methods has the additional benefit, where each patient may experience different values of the outcome and of the covariates over time, of allowing each patient to essentially serve as their own control. This phenomenon was observed in all three studies of the thesis. In Chapter 3, many patients experienced very different urinalysis results as the study progressed, allowing us to observe the same patient in many different states. In Chapters 4 and 5, each patient had adherence measured over the time between two viral load tests. During these time intervals patients exhibited considerable variation in levels of adherence. Similarly here, observing the same patient at many different levels of adherence improves the efficiency of the analysis. The cross-sectional approaches taken in many of the other studies on these topics average adherence over long time periods. How periods of non-adherence are distributed throughout this interval cannot be evaluated when using this method of describing adherence. This illustrates an additional benefit of using longitudinal methods in such studies.

We chose to use the GEE approach to carryout all of the longitudinal data analysis in this thesis. There are other approaches one may use for such problems, including likelihood-based approaches such as random effects models. In the case of a continuous response as in Chapter 3, in particular, random effects models could have easily been used in place of GEE as this is Gaussian regression with random effects for which standard methodology exists. However, because patients were 100% adherent in many of the intervals, the Gaussian parametric distributional assumption inherent in such a random effects analysis did not seem appropriate. This parametric model assumption is not required when inference is done using GEE. In Chapters 4 and 5, random effects approaches also could have been used but methodology for fitting logistic and Poisson regression models involving random effects is not as well developed as in the Gaussian case. Furthermore, in each study described in this thesis, the marginal effects

were of primary interest and estimation of subject-specific effects wasn't necessary to address the scientific hypotheses.

6.3 Future Work

Several hypotheses were proposed in this thesis, and should be subject to further evaluation. In Chapter 3, it was hypothesized those patients testing positive for opiates at the end of the interval were patients relapsing in their opiate addiction, and that an incorrect opiate dose was being used. This hypothesis can be tested by restricting our analysis to those patients who at the beginning of an interval tested positive for opiates. Changes in methadone dose occurring at this time point can then be assessed to see if increasing the dose in these patients increased the level of adherence. Several interesting findings regarding amphetamine poly-substance use were also found. Several possible hypotheses, including some that are supported by animal models or epidemiologic data, were proposed but studies should be conducted to evaluate amphetamine users enrolled in a methadone maintenance program. Specific efforts should be undertaken to understand how poly-substance use in amphetamine users can led to poorer outcomes when compared to users of amphetamine in isolation.

In Chapters 4 and 5, we observed a specific level of adherence which appears to be associated with much higher rates of virologic failure and the accumulation of DRMs. It was hypothesized that this level of adherence is not in itself the cause of such observations but rather that this level of adherence represents a specific pattern of non-adherence which exposes circulating virus to sub-optimal drug concentrations on a frequent basis. This hypothesis can be evaluated by demonstrating that the frequencies of specific patterns of non-adherence are

associated with these two outcomes, independent of adherence. Furthermore, in patients who have experienced multiple lines of HAART therapy or have a significant number of important DRMs, HAART even under the most ideal cases won't be able to achieve viral suppression. In these patients, people have proposed using other outcomes to measure success, including changes in viral load. Although many have fit non-linear longitudinal models to viral load data, these models usually make the dubious assumption that heterogeneous drug concentrations exist, which completely inhibits all viral replication. Drug concentrations are subject to the body's metabolism or elimination processes, which in turn make the level of viral replication fluctuate as a function of time. Although assuming complete replication is suitable in some circumstances, these would be limited to situations where patients are taking their dose *exactly* as prescribed and the drug is completely effective (i.e. no drug resistance). Patients exhibiting any deviations from the regimen's dosing requirement are subject to periods where viral replication is only partially suppressed. Both viral kinetics and pharmacokinetics are governed by fairly well understood mathematical models and it would seem possible to use such models when developing longitudinal frameworks to evaluate specific hypotheses regarding viral load type outcomes.

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