Statistical and Econometric Analysis of the Treatment of HIV/AIDS

Thesis by

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Dedication

To my parents

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Abstract

The epidemic of HIV/AIDS in the United States is constantly changing and evolving, starting from patient zero to now an estimated 650,000 to 900,000 Americans infected. The nature and course of HIV changed dramatically with the introduction of antiretrovirals. This discourse examines many different facets of HIV from the beginning where there wasn't any treatment for HIV until the present era of highly active antiretroviral therapy (HAART). By utilizing statistical analysis of clinical data, this paper examines where we were, where we are and projections as to where treatment of HIV/AIDS is headed.

Chapter Two describes the datasets that were used for the analyses. The primary database utilized was collected by myself from an outpatient HIV clinic. The data included dates from 1984 until the present. The second database was from the Multicenter AIDS Cohort Study (MACS) public dataset. The data from the MACS cover the time between 1984 and October 1992. Comparisons are made between both datasets.

Chapter Three discusses where we were. Before the first anti-HIV drugs (called antiretrovirals) were approved, there was no treatment to slow the progression of HIV. The first generation of antiretrovirals, reverse transcriptase inhibitors such as AZT (zidovudine), DDI (didanosine), DDC (zalcitabine), and D4T (stavudine) provided the first treatment for HIV. The first clinical trials showed that these antiretrovirals had a significant impact on increasing patient survival. The trials also showed that patients on these drugs had increased CD4+ T cell counts. Chapter Three examines the distributions of CD4 T cell counts. The results show that the estimated distributions of CD4 T cell counts are distinctly non-Gaussian. Thus distributional assumptions regarding CD4 T cell counts must be taken into account when performing analyses with this marker. The results also show the estimated CD4 T cell distributions for each disease stage: asymptomatic, symptomatic and AIDS are non-Gaussian. In-

terestingly, the distribution of CD4 T cell counts for the asymptomatic period is significantly below that of the CD4 T cell distribution for the uninfected population suggesting that even in patients with no outward symptoms of HIV infection, there exists high levels of immunosuppression.

Chapter Four discusses where we are at present. HIV quickly grew resistant to reverse transcriptase inhibitors which were given sequentially as mono or dual therapy. As resistance grew, the positive effects of the reverse transcriptase inhibitors on CD4 T cell counts and survival dissipated. As the old era faded a new era characterized by a new class of drugs and new technology changed the way that we treat HIV-infected patients. Viral load assays were able to quantify the levels of HIV RNA in the blood. By quantifying the viral load, one now had a faster, more direct way to test antiretroviral regimen efficacy. Protease inhibitors, which attacked a different region of HIV than reverse transcriptase inhibitors, when used in combination with other antiretroviral agents were found to dramatically and significantly reduce the HIV RNA levels in the blood. Patients also experienced significant increases in CD4 T cell counts. For the first time in the epidemic, there was hope. It was hypothesized that with HAART, viral levels could be kept so low that the immune system as measured by CD4 T cell counts would be able to recover. If these viral levels could be kept low enough, it would be possible for the immune system to eradicate the virus. The hypothesis of immune reconstitution, that is bringing CD4 T cell counts up to levels seen in uninfected patients, is tested in Chapter Four. It was found that for these patients, there was not enough of a CD4 T cell increase to be consistent with the hypothesis of immune reconstitution.

In Chapter Five, the effectiveness of long-term HAART is analyzed. Survival analysis was conducted on 213 patients on long-term HAART. The primary endpoint was presence of an AIDS defining illness. A high level of clinical failure, or progression to an endpoint, was found.

Chapter Six yields insights into where we are going. New technology such as viral genotypic testing, that looks at the genetic structure of HIV and determines where mutations have occurred, has shown that HIV is capable of producing resistance mu-

tations that confer multiple drug resistance. This section looks at resistance issues and speculates, ceterus parabis, where the state of HIV is going. This section first addresses viral genotype and the correlates of viral load and disease progression. A second analysis looks at patients who have failed their primary attempts at HAART and subsequent salvage therapy. It was found that salvage regimens, efforts to control viral replication through the administration of different combinations of antiretrovirals, were not effective in 90 percent of the population in controlling viral replication. Thus, primary attempts at therapy offer the best change of viral suppression and delay of disease progression. Documentation of transmission of drug-resistant virus suggests that the public health crisis of HIV is far from over. Drug resistant HIV can sustain the epidemic and hamper our efforts to treat HIV infection. The data presented suggest that the decrease in the morbidity and mortality due to HIV/AIDS is transient. Deaths due to HIV will increase and public health officials must prepare for this eventuality unless new treatments become available. These results also underscore the importance of the vaccine effort.

The final chapter looks at the economic issues related to HIV. The direct and indirect costs of treating HIV/AIDS are very high. For the first time in the epidemic, there exists treatment that can actually slow disease progression. The direct costs for HAART are estimated. It is estimated that the direct lifetime costs for treating each HIV infected patient with HAART is between $353,000 to $598,000 depending on how long HAART prolongs life. If one looks at the incremental cost per year of life saved it is only $101,000. This is comparable with the incremental costs per year of life saved from coronary artery bypass surgery.

Policy makers need to be aware that although HAART can delay disease progression, it is not a cure and HIV is not over. The results presented here suggest that the decreases in the morbidity and mortality due to HIV are transient. Policymakers need to be prepared for the eventual increase in AIDS incidence and mortality. Costs associated with HIV/AIDS are also projected to increase. The cost savings seen recently have been from the dramatic decreases in the incidence of AIDS defining opportunistic infections. As patients who have been on HAART the longest start

to progress to AIDS, policymakers and insurance companies will find that the cost of treating HIV/AIDS will increase.

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Chapter 1 Introduction

AIDS (acquired immune deficiency syndrome) was first reported by Michael Gottlieb in 1981 [1]. The virus that causes AIDS was first isolated by Barre-Sinoussi in 1983 [2]. Since then approximately 25 million people worldwide have been infected with HIV (human immunodeficiency virus).

Before the advent of effective treatment for HIV/AIDS, the impact of HIV/AIDS on the United States health care system was large. It was estimated that the lifetime cost of treating each HIV-infected individual was $119,000 [3] (1993 dollars). According to the Centers for Disease Control (CDC), 650,000 to 900,000 Americans are living with HIV and at least 40,000 new infections occur each year [4].

Before zidovudine (AZT), the first antiretroviral, there was no treatment for HIV. It was estimated that the average time between seroconversion and diagnosis of an AIDS defining opportunistic infection was ten years [5]. Risk of contracting an opportunistic infection increased greatly once a patient's CD4 T cell counts went below 200 cells/mm^3[1]. Once a patient had a CD4 T cell count less than 200 cells/mm^3, median time until death was two years [6]. AIDS was becoming a public health crisis. There were too few hospital beds, too few doctors, and no treatment to give patients to slow the progression of the disease.

The first anti-HIV drug zidovudine (AZT, retrovir) was first approved by the Food and Drug Adminstration (FDA) in 1986. AZT is part of a class of drugs called reverse transcriptase inhibitors (RTI). RTI's attack the enzyme that allows HIV to replicate, reverse transcriptase. The first clinical trial with AZT showed a marked increase in survival for patients that were on the AZT arm of the study [7]. Patients on AZT also experienced an increase in CD4 T cell counts. Similar results were found with other reverse transcriptase inhibitors. Viral load reductions with RTI's were

[1] CD4 T cells are a primary target of HIV. CD4 T cells are also an important component of the immune system. The normal range is 700-1400 cells/mm^3.

rapid but incomplete and viral rebound was found six to twelve months later. Viral rebound was due to the presence of resistance conferring mutations in HIV reverse transcriptase [8, 9]. The improvements seen with RTI monotherapy were transient and patients experienced a resurgence in viral load, CD4 T cell decline and disease progression.

Protease inhibitors (PI) were the next step forward in the fight against AIDS. Protease inhibitors were different from reverse transcriptase inhibitors in that these drugs attacked a different target of the HIV replication cycle. Thus HIV that was resistant to RTI's would be susceptible to protease inhibitors. RTI's and PI's were used in combination, referred to as highly active antiretroviral therapy (HAART), produced dramatic and sustained reductions in viral load. CD4 T cells increased substantially. CDC estimates of morbidity and mortality due to HIV infection decreased for the first time [4]. This result is attributed to the introduction of HAART [10]. For the first time in the epidemic, there was hope that HIV could be managed as a chronic disease.

It was hypothesized that if viral levels could be kept low enough long enough that the immune system would be able to reconsitutute [11]. Immune reconstitution, that is bringing the levels of CD4 T cell counts back up to levels similar to uninfected people, would reduce the risk of patients contracting an opportunistic infection. Failure to isolate infectious virus from PBMC (peripheral blood mononuclear cells) or lymphoid tissue from patients who had sustained viral suppression on HAART for two years [12] fueled speculation that HIV could be eradicated [13]. In recent studies, it was found that replication competent virus could be isolated from a latent reservoir [14, 15, 16]. These results suggest that at present, HIV is not eradicated by HAART.

Chapter Two details the datasets that were used for the analyses. The primary dataset utilized was collected by myself from Dr. Michael Gottlieb's outpatient clinic. Outpatient clinics differ substantially from the controlled environment of a clinical trial. To assess the impact of HIV and its treatment on the more general patient population, it was necessary to obtain data from an outpatient clinic. Data were

collected between June 1995 and February 1999. Data were abstracted from patient charts and entered into a computer. Insurance information was then matched into the clinical database by a unique identification number. Care was taken to ensure patient privacy and anonymity. Diagnostic tests were run to ensure the accuracy of the data. Any discrepancies were remedied by checking the chart or consultation with the treating physician. Data were collected on biologic markers such as CD4 T cell counts and percentages, CD8 T cell counts and percentages, ratio of CD4 T cell counts to CD8 T cell counts, HIV-1 RNA levels (viral loads), $\beta 2$ microglobulin levels, presence of p24 antigen, cholesterol, triglycerides, and weight. Data was also gathered on cost, insurance status, diagnosis, treatments, antiretroviral therapy, prophalaxis therapy, home health care, and hospitalizations.

The second data set was obtained from the Multicenter AIDS Cohort Study (MACS) public data set. The MACS is described Kaslow et al. [17]. The MACS is a prevalence database that has both seropositive (HIV infected) and seronegative (HIV uninfected) in the study population. Data was gathered on CD4 T cell counts, CD8 T cell counts, physical examination findings, and survey questions on medications, diagnoses, and socio-economic questions. Data from the public data tape start in 1984 and end October 1992.

Chapters Three describes where we were, no treatment to reverse transcriptase inhibitor dual or monotherapy. It was found that reverse transcriptase inhibitors increased CD4 T cell counts. However, this effect was transient. It was also assumed that the distribution of CD4 T cell counts was Gaussian, regardless of the disease stage. A nonparametric density estimator was applied to the repeated CD4 T cell counts of patients on reverse transcriptase inhibitor mono or dual therapy. In section three it is shown that this methodology applied to repeated measures data is consistent and unbiased. The analysis found that the estimated distribution of the CD4 T cell counts were not distributed Gaussian. It was also found that when the patients were segregated by disease stage: asymptomatic, symptomatic, and AIDS, the CD4 T cell counts in all stages were distinctly non-Gaussian. In fact, in the AIDS stage the distribution is bimodal. In the asymptomatic period, there was a substantial

decrease in CD4 T cell counts when compared to the uninfected population. This suggests that patients who have no outward symptoms of HIV could have a high level of immunosuppression despite antiretroviral therapy.

Chapters Four and Five detail where we are presently in the treatment of HIV infection. HIV quickly grew resistant to dual and mono therapy reverse transcriptase inhibitors. Protease inhibitors and combination antiretroviral therapy rapidly and significantly reduced the levels of circulating virus in HIV-infected patients. Increases in CD4 T cell counts and reductions in the morbidity and mortality due to HIV lead scientists to hypothesize about immune reconstitution and viral eradication. Two papers are presented in that address both of these hypotheses. Chapter Four looks at the CD4 T cell distribution of patients on highly active antiretroviral therapy (HAART). Using a nonparametric density estimator, it is found that the CD4 T cell increases due to HAART are insufficient to be consistent with the hypothesis of immune reconstitution. The paper also shows that the estimator is consistent and unbiased for repeated measures data. Chapter Five looks at the rate of clinical failure for patients on long- term HAART. Clinical failure is defined here as diagnosis with an opportunistic infection or a decrease of CD4 T cell counts to below 200 cells/mm^3. It was found that the median time until clinical failure was 586 days. Failure of HAART to eradicate the virus leaves patient vulnerable to viral resistance and resurgence.

Chapter Six glimpses at the future of HIV treatments. A growing number of clinical patients are experiencing virologic and clinical failure despite being on HAART. Virologic failure is defined as failure to keep the virus suppressed to very low levels in the peripheral blood and plasma. Clinical failure is defined as progression to an AIDS defining event. As more and more patients are failing their primary attempts at combination therapy, salvage therapy, defined as different combinations of drugs to which the patient is naive, has become an important issue. Whether due to cross-resistance or viral breakthrough, it was found that salvage therapy is not effective in producing a sustained virologic response in heavily pre-treated patients. Within 6 months after the initiation of a salvage regimen, the majority of patients experience virologic failure. This disappointing result is due, for the most part, to drug resistant

virus. Viral genotyping has shown high level resistance in patients who fail salvage regimens. With the documented transmission of drug resistant strains of HIV, the scenario worsens. If the prevalence of resistant strains is high, then patients may be already resistant to antiretroviral medications. Because of cross-resistance, there might not exist an antiretroviral agent to which there would not be some form of resistance.

Chapter Seven looks at the economic costs related to the treatment of HIV. The direct and indirect costs of treating HIV/AIDS are very high. For the first time in the epidemic, there exists treatment that can actually slow disease progression. The direct costs for HAART are estimated. It is estimated that the direct lifetime costs for treating each HIV infected patient with HAART is between $546,668.78 to $723,069.20 depending on how long HAART prolongs life. If one looks at the incremental cost per year of life saved it is only $150,948.84. This is comparable with the incremental costs per year of life saved from coronary artery bypass surgery.

Policy makers need to be aware that although HAART can delay disease progression, it is not a cure and HIV is not over. The results presented here suggest that the decreases in the morbidity and mortality due to HIV are transient. Policymakers need to be prepared for the eventual increase in AIDS incidence and mortality. Costs associated with HIV/AIDS are also projected to increase. The cost savings seen recently have been from the dramatic decreases in the incidence of AIDS defining opportunistic infections. As patients who have been on HAART the longest start to progress to AIDS, policymakers and insurance companies will find that the cost of treating HIV/AIDS will increase.

Chapter 2 Data

2.1 Outpatient Data

The primary dataset used in the analyses was constructed from the retrospective chart review of 325 charts of HIV-infected patients from Dr. Michael Gottlieb's outpatient clinic. Data collection and follow up was performed continuously over the space of five years. Patients were selected by a 30 percent random sample of Gottlieb's patient base. The only selection criteria was seropositivity. No patients were excluded because of this criteria. As this was an intent to treat analysis, all variables were gathered on all patients regardless of treatment regimen.

Data were collected by reading through patient charts and entering the data into Excel 5.0 on a laptop computer. Excel was chosen for its flexibility and ease into importing into a variety of statistical packages. As technologies and treatments for HIV changes, it was simple to change the Excel format. Financial data was merged into the clinical database by patient unique identification number. Care was taken to ensure patient privacy and anonymity. Follow-up on patient disease progression and clinical markers was performed every four months. Data was gathered on clinical markers such as CD4 T cell count and percentage of lymphocytes, CD8 T cell count and percentage of lymphocytes, ratio of CD4 T cells to CD8 T cells, HIV RNA levels (viral load), viral genotypic mutations, $\beta 2$ microglobulin, p24 antigen, cholesterol, triglycerides, and weight. Data was also gathered on diagnosis, history of opportunistic infections, antiretroviral medication history, treatments, disease stage, prophalaxis medications, and compliance with drug regimens. As patients enter the doctor's office at different intervals as well as have lab tests performed at different intervals, all data were entered as date of visit or laboratory test and result. As a result, some patients have more test results than others.

Compliance was measured as a binary variable. In the course of the doctor visit,

the doctor would ask the patient if they took all of their medication as prescribed. If the answer was negative, the date was marked as a one for noncompliance on that date, otherwise noncompliance was coded as a zero. Disease stage was coded as a discrete variable coupled with a date: zero for asymptomatic, one for symptomatic, and two for AIDS and the date of the assessment. Patients who were coded as asymptomatic for a particular date had no outward symptoms of HIV infection. Patients who were coded as symptomatic had increased symptoms of immune dysfunction. Symptoms included oral candidiasis, night sweats, and recurrent herpes zoster. Patients were defined as having AIDS if they were diagnosed with an opportunistic infection. Note that there is monotonicity in the ordering of the disease stages. Once a patient was coded as being in a certain stage, the same patient could not revert to an earlier stage. For example, if a patient is coded as having AIDS, they could never revert back to being symptomatic.

CD4 T cell counts and percentages, CD8 T cell counts and percentages, $\beta 2$ microglobulin, p24 antigen, cholesterol, triglyceride levels, HIV RNA levels, and weight were all coded as continuous variables with their corresponding dates. Diagnoses were coded as their ICD-9 numeric code as is standard in insurance documentation. Antiretroviral and other treatment medications were coded as a date and a binary variable that was one if the patient was on that particular drug at that date. Economic and demographic variables such as cost, age, gender, insurance status, and disability status were also collected. Cost data represents the charge to the patient or their respective insurance company.

In the cohort, there are 28 females and 297 males. The majority of men in the cohort were infected by homosexual contact. The women were infected by heterosexual contact. The median age is 42 years (range 21-69 years). In the sample, 78 percent of the patients are privately insured, 16 percent are covered by Medi-Care or Medi-Cal, and 11 percent belong to health maintenance organizations or have no insurance.

Patients were heterogeneous with respect to disease stage. Roughly 33 percent of the patients came into the clinic asymptomatic. These patients have no outward symptoms of HIV disease. One-third were symptomatic. These patients are showing

signs of increase immune suppression but have not experienced an opportunistic infection. Oral candidiasis and recurrent herpes zoster are indicative of symptomatic HIV infection. One-third of the patients entered the study already having experienced an opportunistic infection. These patients have severe immune depletion and are at increased risk of death. Note that AIDS is not classified based on absolute CD4 T-cell count, only on the presence of an AIDS defining opportunistic infection. The reason for this coding will become apparent in the section on nonparametric density estimation of CD4+ T-cell counts by disease stage.

The patients in the study are homogeneous with respect to financial status. Most patients in the study are privately insured and well off. These patients generally are well educated and well informed about their disease. These patients, for the most part, can afford combination antiretroviral therapy. The vast majority of the patient in this cohort are on antiretroviral medication. In the era of HAART, 90 percent of the patients sampled were on combination antiretroviral therapy. The culmination of these factors is important because it relates to the issue of compliance to the antiretroviral regimen. These patients are well educated about the various aspects of the disease and are warned about the ramifications of drug resistance due to noncompliance. Studies have shown that patients who are poorer or who are intravenous drug users are more likely to be noncompliant with the complicated regimens required for highly active antiretroviral therapy (HAART). Since this patient group consists primarily of well educated gay white males, one could argue that this specific population would be more likely to be compliant with the therapeutic regimens.

The question of representativeness is intrinsic to disease cohorts [18]. The question of selection bias is especially relevant in a cohort from one outpatient clinic. The fact that 73 percent of the population is privately insured and only one percent of the population are IV drug users suggests that it is not. However, this population can serve as the frontier of medical technology and HIV care. Michael Gottlieb is renowned as an HIV specialist. He is credited with the first diagnosis of AIDS in the United States and for finding the correlation between CD4 T-cell counts and HIV. He

has been on some of the earliest clinical trials and continues to be involved in clinical trials and expanded access programs. He utilizes the most advanced technologies such as genotypic and phenotypic testing. Thus his patients receive the most advanced care medicine can offer. The patient population that he serves offers the best chance for compliance in that they are well educated and motivated to be compliant with their antiretroviral regimens and are aggressive in the treatment of their disease. In this way, this study looks at the frontier of HIV treatment in the outpatient setting. Trends in this population will be seen first and then later in a more representative population. This study should be viewed in that light.

Outcomes from an outpatient clinic can differ substantially from clinical trials. Clinical trials are highly controlled experiments that test whether one treatment regimen is more effective than another. Patients are carefully selected to be homogeneous with respect to disease status and other factors deemed important to the trial. Patients are also monitored very closely. Subjects who are non-compliant are not included in the study and subjects are required to stay on the same treatment for the prescribed length of time. In the more general clinical setting of the doctor's office, patients enter treatment at different disease stages. Patients are often heterogeneous in many different respects. Patient are more likely to switch treatment regimens and are more likely to be noncompliant when compared to the clinical trial population. Procedures such as viral load (quantitation of the number of HIV RNA copies/ml) and CD4+ T-lymphocyte quantitation are done at different frequencies for patients depending on disease state, insurance type and other factors. Data of this nature are inherently more complex to analyze but also can yield deeper insights into the impact of HIV on the more general outpatient population of HIV infected individuals. Because of this complexity, standard statistical techniques are often insufficient.

The analytic approach to the dataset that I collected is an intent-to-treat analysis. Although a compliance rate is measured, patients who are noncompliant are not dropped from the analysis. The non-compliance rate for this cohort of patients is 30 percent which is significantly lower than non-compliance rates found in other studies. In the clinical setting, patients may be noncompliant to their therapeutic regimen

for a variety of reasons: difficulty of regimen, side-effects, or toxicity. To evaluate the efficacy of a drug, clinical trials only analyze patients who were compliant to the treatment. In the doctor's office results may differ from those seen in a clinical trial because there are less stringent controls. To assess the clinical effectiveness of antiretrovirals, I conduct intent-to-treat analyses. This should yield results consistent with those that physicians are seeing in their outpatient clinics.

2.2 MACS Data

Data were obtained from the Multicenter AIDS Cohort Study (MACS) public dataset. The MACS is a prevalent cohort that collects data from volunteers at five separate centers. The original cohort consisted of 4,954 gay and bisexual men. Follow up was every six months. Each visit consisted of physical exams, laboratory tests and questionnaires about their condition, treatments, and history of opportunistic infections. The cohort was started in 1984, before HIV was isolated. Thus the MACS data contains seronegative patients as well as seroconverters. From April 1987 and September 1991, the cohort was opened to include minorities, women and partners of members of the original cohort. In 1990, visits 14-17, questionnaire on health care cost and utilization were given. The details of the characteristics of the MACS are well known in the literature [17]. The public data set covers MACS visits 1-17, which covers the start of the MACS until October 1, 1992. Subjects in the MACS cohort were on antiretroviral treatment ranging from no treatment to mono or dual therapy with reverse transcriptase inhibitors. As the data end in 1992 and protease inhibitors were approved in 1995, data on protease inhibitors in this population were not available on the MACS public data set.

Cohort studies are especially useful for describing the natural history of HIV/AIDS. In these studies, therapeutic interventions are not given according to a protocol; they are given to patients on an individualized basis by their outpatient providers. Clinical trials evaluate the efficacy of a specific treatment versus a placebo or a standard of care. Cohort studies can be used to estimate effectiveness of a treatment. That

is, the effectiveness of a treatment on the population for which it was intended in reducing morbidity and mortality due to the illness. Data gathered from these cohort studies cannot be readily extrapolated to the general population without accounting for selection bias. Inherent biases occurs in these cohort studies when the population being studied is not representative of the more general population. This is often the case with cohort studies. In HIV, most studies are under-represented by women, minorities and intravenous drug users. This fact must be taken into account when extrapolating results from the smaller population to the general population.

The MACS is a significantly larger database and has a more diverse patient population than the database I collected. The dataset that I have collected is clearly too homogeneous to be representative of the HIV infected population. However, I argue that it is homogeneous in an informative way. The population which I study is very well educated and well off. These patients are more likely to receive and afford the latest treatments and technologies and are more likely to be compliant. These patients represent the frontier of HIV therapy and treatment. Trends in treatment are more likely to appear in this population before the more general population. To that end, this population is interesting and worth studying.

Chapter 3 Estimated Distributions of CD4 T Lymphocytes in HIV-1 Infected Patients

Abstract

In the literature, it is standard to assume normality or log normality for CD4 T cell counts. To test this assumption, a nonparametric density estimator was applied to two different datasets. The first dataset is from the Multicenter AIDS Cohort Study (MACS). The second dataset consists of clinical data obtained from an outpatient clinic. The second dataset was sufficiently detailed to separate patients and their corresponding CD4 T cell counts by disease stage: asymptomatic, symptomatic, and AIDS. For each stage, a density estimate was constructed for the CD4 T cell counts. It was found for all disease stages that CD4 T cell counts are not distributed Gaussian. It was also found that even in the asymptomatic stage, there was a significant decrease in CD4 T cell counts as compared to the uninfected population. Thus even if a patient has no outward symptoms of HIV infection, they could be immunodeficient. This suggests that patients should seek treatment early in infection and emphasizes the need for more widespread testing.

3.1 Introduction

In the HIV (human immunodeficiency virus) literature, it is standard to assume normality or log-normality for CD4 T cell distributions [19, 20, 21]. To test this assumption, a nonparametric density estimator is utilized to estimate the distributions of CD4 T cell counts. Two datasets are used. The first dataset comes from the Multicenter AIDS Cohort Study (MACS). The second database consists of data gathered from an outpatient clinic.

The MACS is a large prevalence database that consists of almost 5,000 participants who were at risk for HIV/AIDS at five different sites. Every six months data were obtained on CD4 T cell counts as well as other clinical markers. The second dataset consists of data from 77 patients chosen at random from an outpatient clinic. Data were gathered on CD4 T cell counts as well as other factors in roughly 6 month intervals. The MACS is a larger, more broad study than the data collected from a private clinic. The data from the private clinic, however, is more detailed and can yield insights into clinical progression under various treatment regimens.

In examining CD4 T cell counts, the analysis is often complicated by non-normality and bimodality. Comparisons of populations and levels are not straightforward in these cases. Assumptions of normality, in this case, could lead to biased and misleading results. Nonparametric density estimates were constructed using both cohorts. Estimates were also constructed by disease stage: asymptomatic, symptomatic, and AIDS. The resulting CD4 T cell distributions are compared using Kolmogorov-Smirnov (KS). Kolmogorov-Smirnov is used because it is nonparametric and searches over the entire density. Nonparametric tests are preferable especially when there exists non-normality, skewness, and bimodality.

For both datasets, the MACS and the outpatient dataset, it was found that the estimated CD4 T cell counts were not distributed Gaussian. Estimates of the CD4 T cell distributions for each disease stage were also non-Gaussian. In fact, for the AIDS stage, the distribution was bimodal in the MACS population. This result must be taken into account when making distributional assumptions about CD4 T cell counts.

It was also found that even in patients who are asymptomatic, that is have no outward symptoms of HIV infection, there existed a high degree of immunosuppression of CD4 T cell counts. This suggests that patients should seek treatment early in the course of infection to prevent further immune damage and also emphasizes the need for more widespread testing.

3.2 Data

The methodology discussed in the previous section was applied to data from the Multicenter AIDS Cohort Study (MACS) public data set and to data from a private HIV clinic. The MACS is a prevalent cohort that collects data from volunteers at five separate centers. For each visit there was a physical examination, questionnaires and lab tests. The public data set starts at the beginning of the MACS in 1984 and ends October 1, 1992 and covers MACS visits 1-17. Subjects in this cohort were on therapies ranging from no treatment to combination nucleoside analogue therapy. The data end in 1992; consequentially, there are no subjects on protease inhibitors. The only selection criteria for the data utilized in this analysis, was seropositivity. Observations of CD4+ T-lymphocyte counts on seropositive people yielded 2,166 patients with 19,998 person-visit observations[1]. Approximately 12.2 percent of these observations came from AIDS patients and 87.8 percent came from observations from subjects who did not have an AIDS defining illness.

A separate dataset that was smaller but more detailed than the MACS was collected from a private outpatient clinic that specialized in HIV care. Patients were selected by a ten percent random sample of all the patients in the practice. The only selection criteria was seropositivity[2]. In all 77, patients were included in the study. Data were gathered on clinical markers such as CD4 and CD8 T cells counts, $\beta 2$ microglobulin, p24 antigen and weight. Data were also gathered on disease stage, physician diagnosis, antiretroviral treatment, insurance status, and cost. The data

[1]Patients had 1 to 17 CD4 observations.

[2]Seropositivity is defined as the presence of antibodies to HIV.

covered the years 1984 to 1994. This dataset is important because it offers more detail on patient's disease progression and treatments. Because of this detail it was possible to stratify the patients by disease stage: asymptomatic, symptomatic, and AIDS[3]. Approximately 33 percent of the patients were classified as asymptomatic, 26 percent were symptomatic and 41 percent had experienced an AIDS defining opportunistic infection.

3.3 Analysis

Repeated CD4 measurements on the same person cannot be considered independent [18]. This suggests that a pooled sample of CD4 counts would have some degree of correlation. To ascertain the effects of the correlation on the distribution, an independent sample was created by randomly sampling one CD4 observation per person. This sample is independent, and the standard errors can be calculated in the usual fashion; it is, however, inefficient because it does not make use of all the data. It has also been shown that subsampling non-independent but stationary data can only result in poorer estimators [22, 23]. An independent subset of the MACS was created by taking a random subsample of one CD4 observation from each person. This resulted in a dataset of 2,166 independent observations. The nonparametric density distribution of the independent sample was estimated (Figure 1). The estimated mean was 474.56 cells/mm^3, the standard deviation was 306.02 cells/mm^3, and the standard error of the mean was 6.60 cells/mm^3. The estimation procedure was repeated using the full sample of 19,988 observations (see Figure 2). The mean was 483.27 cells/mm^3, the standard deviation was 298.49 cells/mm^3. After 10,000 bootstrap replications the standard error of the mean was 5.70 cells/mm^3. The two distributions were then compared nonparametrically using the Kolmogorov-Smirnov (KS) test (see Table 1). The asymptotic KS statistic (KSa) was 1.35 and the prob $>$ KSa was greater than 0.05. Therefore, the two distributions are not significantly different at the alpha equals

[3]I use the 1987 CDC definition that defined AIDS as an specified opportunistic infection without regard to the level of CD4 T cells.

0.05 level. The nonparametric distribution was then plotted against the Gaussian curve. By inspection of the curves, one can see there exists a definite skew in the nonparametric distribution. A box plot of the data confirms this, see Figure 2. This suggests that the CD4 distribution of HIV infected patients may not be Gaussian. The distribution was then tested against the hypothesis of normality [24]. The hypothesis of normality was rejected $p < 0.001$ by KS. The Gaussian distribution does not present a good fit for the infected population due to the skewness and truncation at zero. Several other parametric distributions such as the log normal, exponential and Weibull were fit to the distribution with less success. One must take this result into account when comparing different populations of HIV infected individuals and comparing the HIV population to the uninfected population. The Kolmogorov-Smirnov test is completely nonparametric and searches the entire distribution, and thus appropriate for this type of situation. The square root transformation is applied to the CD4 counts, as standard in the literature [18, 25, 26, 27] and the CD4 density was reconstructed and given in Figure 3. The mean of this distribution is 20.74 with bootstrapped standard error 0.135. Using the Kolmogrov-Smirnov test for normality, the hypothesis that the estimated distribution is distributed Gaussian is rejected at p=0.01.

To further illustrate the necessity of testing the normality assumption when constructing estimates, the nonparametric density distribution of CD4 counts was constructed using only observations that came from patients diagnosed with AIDS. The square root transformation is performed to normalize the data. The mean of this distribution is 13.62 with a bootstrapped standard error of the mean 0.164. Figure 4 shows that for the AIDS population from the MACS data, not only is the resulting distribution non-Gaussian (p=0.0001), it is bimodal. The bimodality in the AIDS stage is important to consider when constructing hypothesis tests that rely on underlying normality. It is also important to consider when comparing means and variances of CD4 counts in the AIDS stage.

3.3.1 MACS Cohort Compared With the Outpatient Cohort

The nonparametric density of CD4 T cell counts was estimated utilizing the entire sample from the outpatient data. The mean for this distribution was 365.42 cells/mm^3. After 10,000 replications of the bootstrap, the standard error of the mean was estimated to be 27.52 cells/mm^3. The shape of the distribution is similar to that of the MACS CD4 T cell distribution; see Figure 5. The distribution was then tested against the hypothesis of normality by KS. Normality was rejected at p=0.001. The MACS CD4 T cell distribution was then compared to the estimated CD4 T cell distribution from the outpatient dataset by Komologorov-Smirnov. They were found to be statistically different at the p=0.001 level; see Table 2. The larger standard errors are more than likely due to the smaller sample size of the outpatient data. The difference in the means can possibly be explained by the fact that the MACS population has only 12.2 percent of the CD4 T cell observations from the AIDS disease stage, whereas the outpatient database has 41 percent of the observations from the AIDS stage. As the likelihood of AIDS increases as CD4 T cells decrease [6], this could bias the mean downward.

Since the overall distribution of CD4 T cells can be viewed as a mixture of all of the distributions from each disease stage, the nonparametric CD4 T cell density distribution was constructed for each disease stage. The outpatient database is sufficiently detailed to segregate patients into all three disease stages. The MACS could only be separated into observations from the AIDS stage and observation from patients without AIDS (the noAIDS distribution). The CD4 T cell distribution from AIDS observations from the MACS had a mean of 251.07 cells/mm^3 with bootstrapped standard error of the mean 11.98 cells/mm^3; see Figure 6. The AIDS CD4 T cell distribution from the outpatient dataset had a mean of 167.92 cells/mm^3 with bootstrapped standard error of 28.37 cells/mm^3; see Figure 7. The hypothesis that the two distributions are the same is rejected by KS at p=0.001 level; see Table 3. The noAIDS CD4 distribution from the MACS had a mean of 409.21 cells/mm^3 with a bootstrapped standard error of the mean of 6.49 cells/mm^3; see Figure 8. The

noAIDS CD4 distribution from the outpatient data had a mean of 450.11 cells/mm^3 with bootstrapped standard error of 28.62 cells/mm^3; see Figure 9. Standard ANOVA fails to reject the hypothesis that the two distributions are the same, p=0.3421, while KS rejects with p=0.040. This illustrates the necessity of taking into account the underlying distributional assumption, especially in small samples.

3.3.2 Densities by Disease Stage

The outpatient data was sufficiently detailed to separate patients into three distinct disease stages: asymptomatic, symptomatic, and AIDS. AIDS was defined as diagnosis with an opportunistic infection regardless of CD4 T cell count. Patients who were coded as symptomatic had increasing symptoms of HIV infection but were not diagnosed with an opportunistic infection. Symptoms included oral candidiasis, night sweats, and recurrent herpes zoster. Patients who were considered asymptomatic had no outward symptoms of HIV infection. The AIDS CD4 distribution had a mean of 167.92 cells/mm^3 with bootstrapped standard error of 28.37cells/mm^3. KS tests of normality rejected the hypothesis that the estimated distribution was Gaussian at p=0.001. The mean for the symptomatic population was 394.00 cells/mm^3 with a bootstrapped standard error of the mean of 30.74 cells/mm^3. The shape of the distribution was roughly normal with a distinct skew; see Figure 10. The hypothesis of normality was rejected by KS at p=0.001. The mean of the asymptomatic CD4 T cell distribution was 550.0 cells/mm^3 with a bootstrapped standard error of the mean of 51.62 cells/mm^3. The estimated distribution has a definite skew and was bimodal; see Figure 11. The hypothesis of normality is also rejected by KS at p=0.001. One might expect that the CD4 T cell distribution of asymptomatic patients would be similar to CD4 distribution of the uninfected population. However, it was found that this was not the case. The distribution of CD4 T cell counts for the uninfected population is N(1017,329) [28]. The asymptomatic CD4 distribution is significantly different than the uninfected population by KS at the p=0.001 level. This suggests that HIV-infected patients who have no outward symptoms could still have a high de-

gree of immunosuppression. The results also strongly suggest that CD4 T cell counts are not distributed Gaussian in HIV infected patients.

3.4 Discussion

In both datasets it was found that the estimated CD4 T cell distributions were distinctly non-Gaussian. Thus modeling CD4 T cell counts distributions, trajectories and other analyses utilizing CD4 T cell counts must take this result into account. If normality is not a valid assumption, the nonparametric techniques should be considered. Failure to take distributional assumptions into account can lead to biased or misleading results.

It was also found that the CD4 distribution from the MACS were significantly different from the outpatient dataset. This may be due to the relatively small sample size of the outpatient data or an artifact of selection bias. There could also exist something systematically different about the outpatient dataset. However, in both datasets the CD4 distributions were all non-Gaussian.

The fact that there was a high degree of CD4 T cell depletion in the asymptomatic stage suggests that there exist a high degree of immune dysfunction even in patients who have no outward symptoms. This suggests that patients should seek treatment early in their disease course to prevent further immune system damage. The results also emphasize the need for more widespread testing.

3.5 Figures

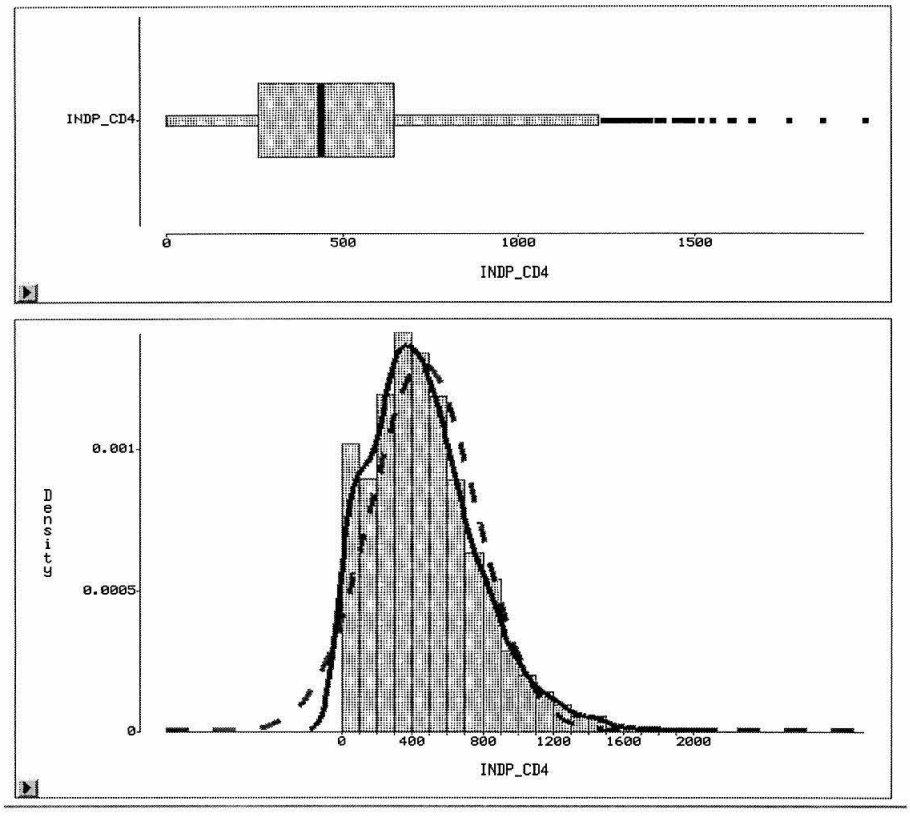

Figure 3.1: a. Box plot of 2,166 independent CD4 T cell draws from the MACS. b. Solid line: nonparametric density of CD4 T cell counts estimated from independent draws from the MACS. Broken line: data fitted to the Gaussian distribution.

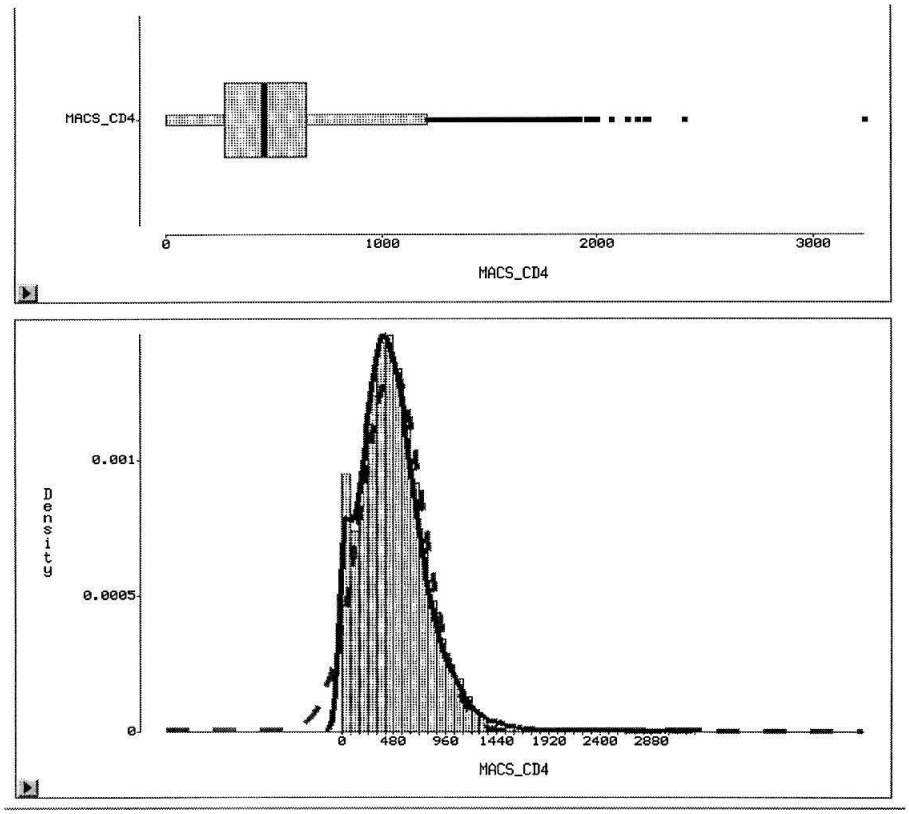

Figure 3.2: a. Box plot of 19,988 CD4 T cell observations from the MACS. b. Solid line: nonparametric density estimate of the CD4 T cell counts from the MACS. Broken line: data fitted to the Gaussian distribution.

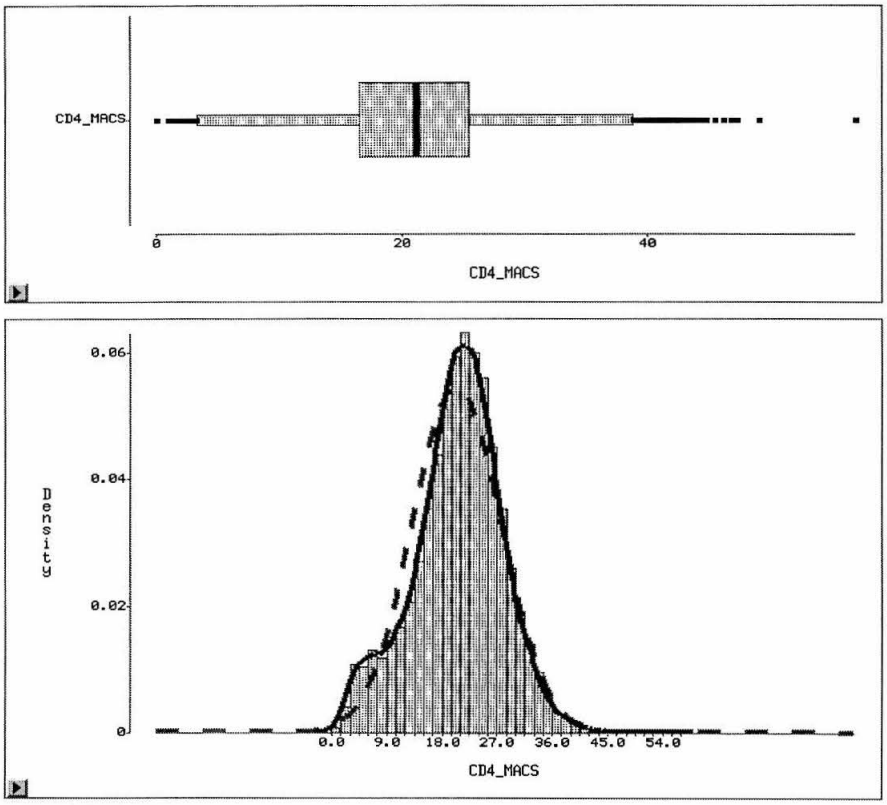

Figure 3.3: a. Box plot of square root CD4 T cell observations taken from the MACS. b. Solid line: nonparametric density estimate of CD4 T cell counts taken from the MACS. Broken line: data fitted to the Gaussian distribution.

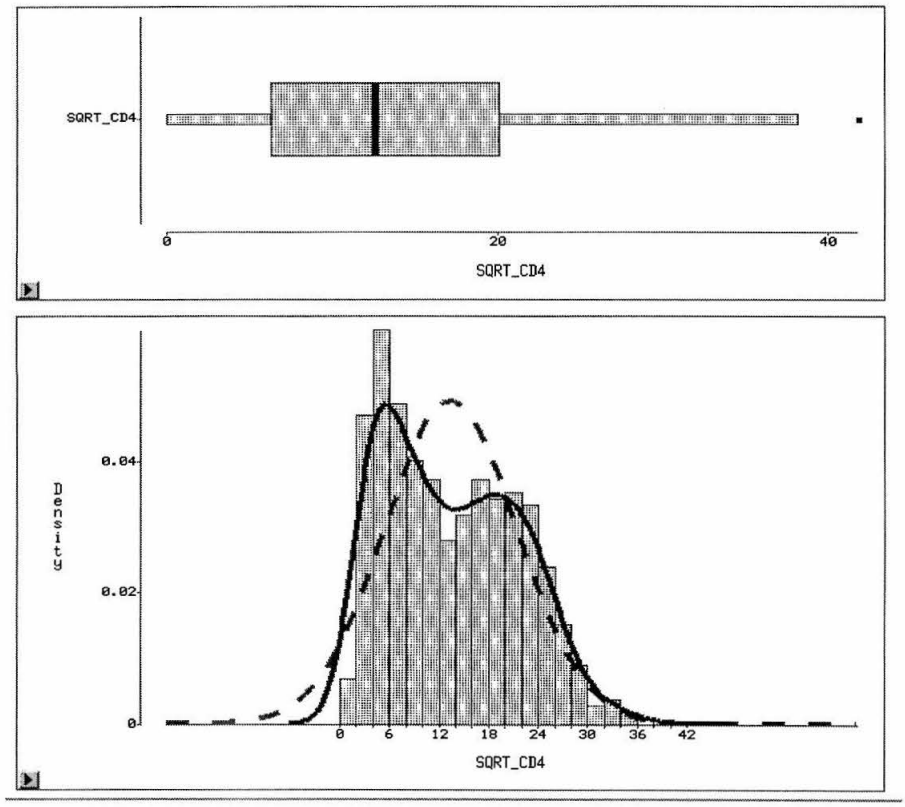

Figure 3.4: a. Box plot of square root CD4 T cell observations taken from the AIDS stage of the MACS. b. Solid line: nonparametric density estimate of CD4 T cell counts taken from the AIDS stage of the MACS. Broken line: data fitted to the Gaussian distribution.

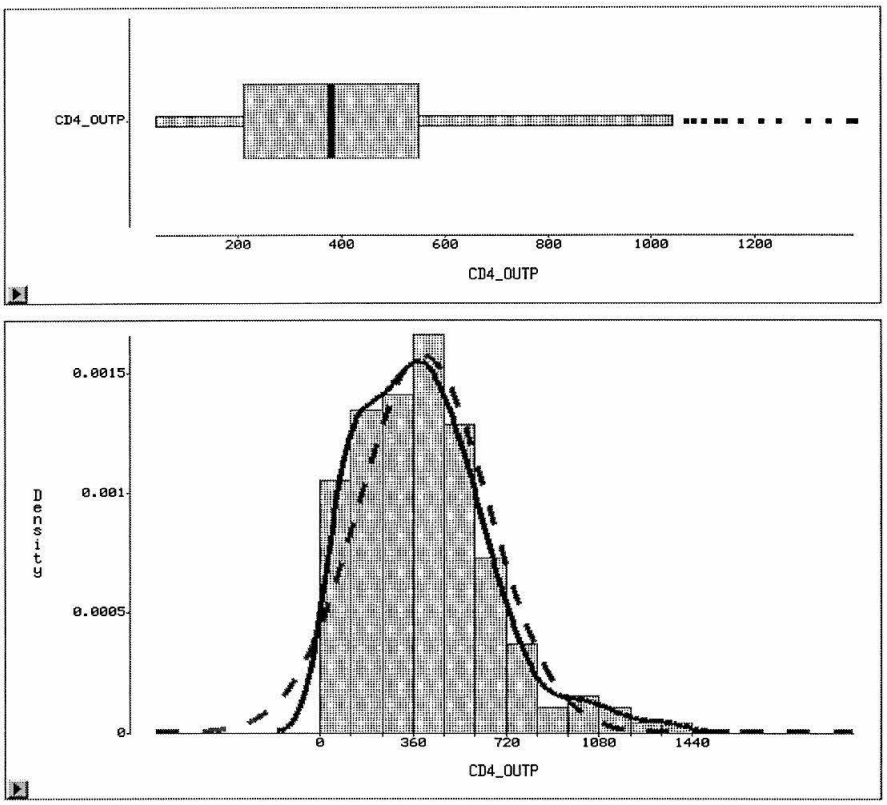

Figure 3.5: a. Box plot of 600 CD4 T cell observations from the outpatient dataset. b. Solid line: nonparametric density estimate of CD4 T cell counts from the outpatient dataset. Broken line: data fitted to the Gaussian distribution.

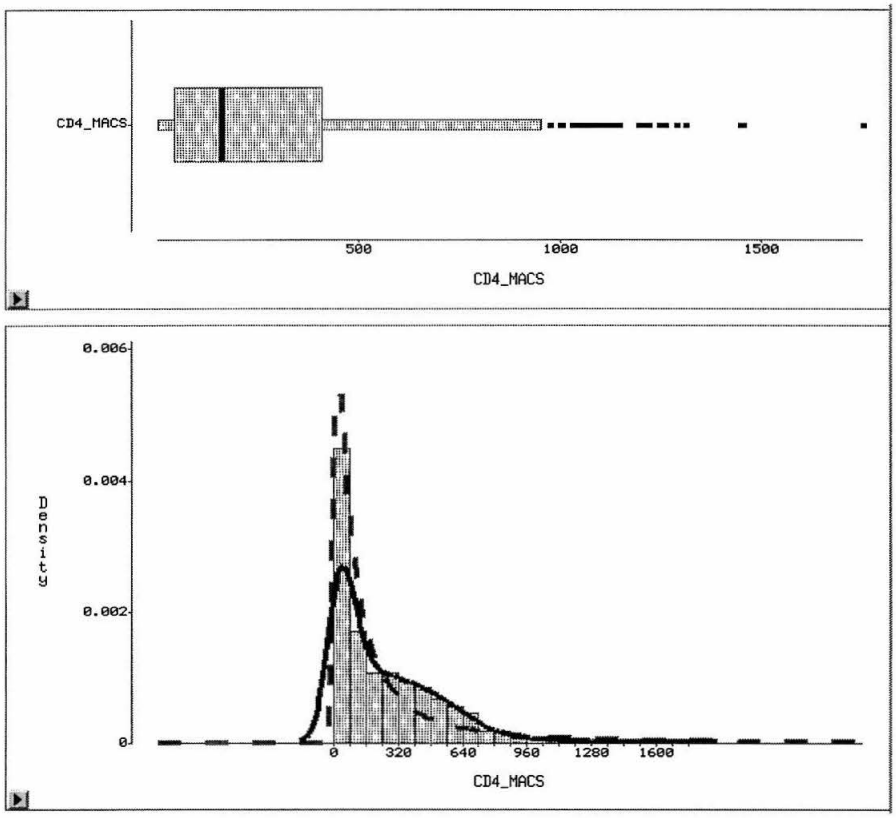

Figure 3.6: a. Box plot of CD4 T cell counts taken from the AIDS stage of the MACS. b. Solid line: nonparametric density estimate of CD4 T cell counts taken from the AIDS stage of the MACS. Broken line: data fitted to the log normal distribution.

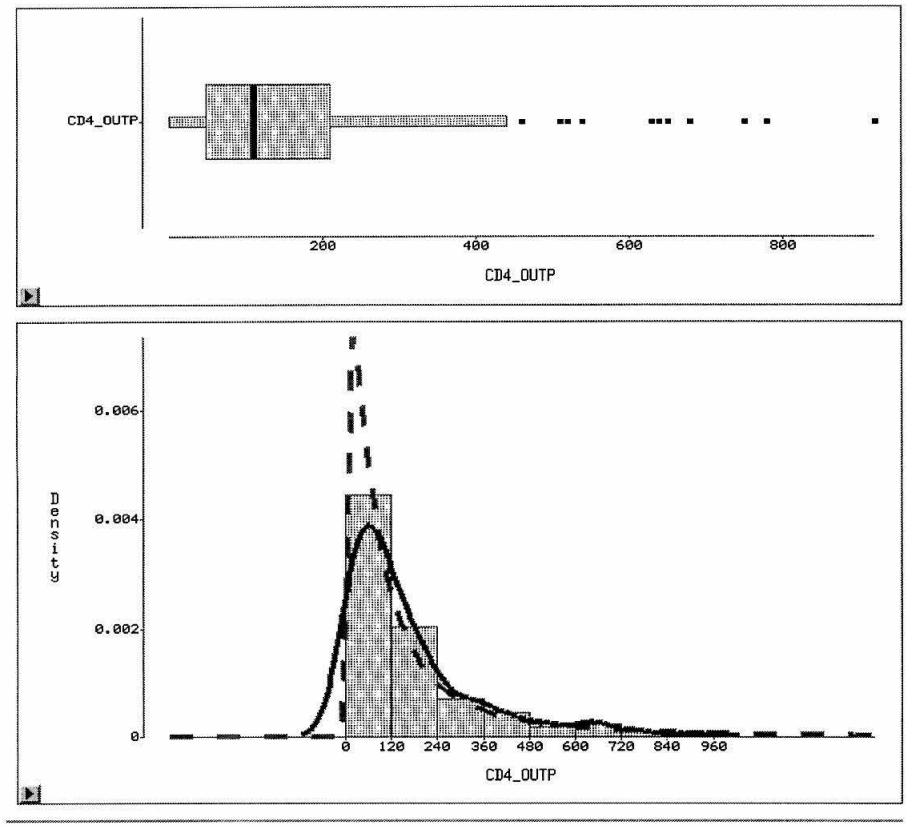

Figure 3.7: a. Box plot of CD4 T cell counts taken from the AIDS stage of the outpatient dataset. b. Solid line: nonparametric density estimate of CD4 T cell counts taken from the AIDS stage of the outpatient dataset. Broken line: data fitted to the log normal distribution.

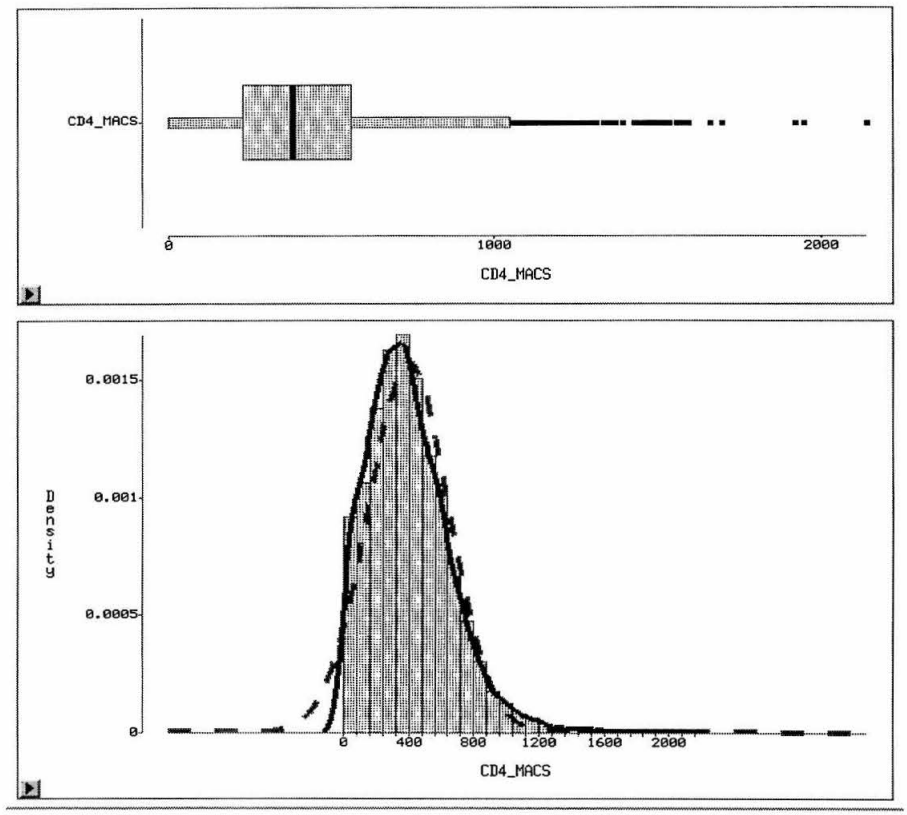

Figure 3.8: a. Box plot of CD4 T cell counts taken from patients without AIDS from the MACS. b. Solid line: nonparametric density estimate of CD4 T cell counts taken from patients without AIDS from the MACS. Broken line: data fitted to the Gaussian distribution.

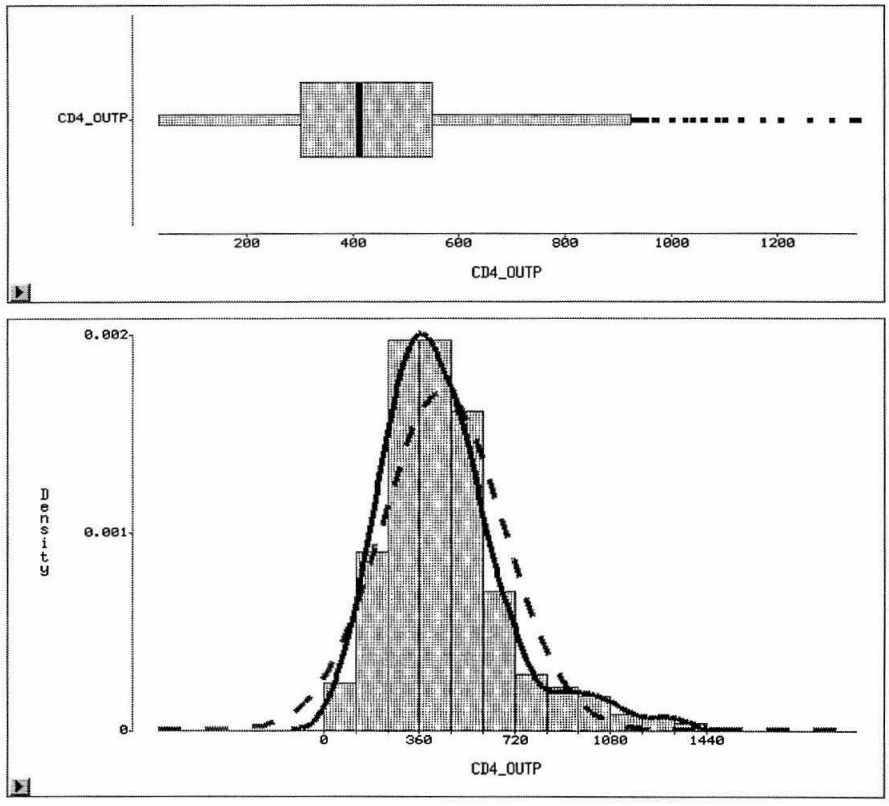

Figure 3.9: a. Box plot of CD4 T cell counts taken from patients without AIDS from the outpatient dataset. b. Solid line: nonparametric density estimate of CD4 T cell counts taken from patients without AIDS from the outpatient dataset. Broken line: data fitted to the Gaussian distribution.

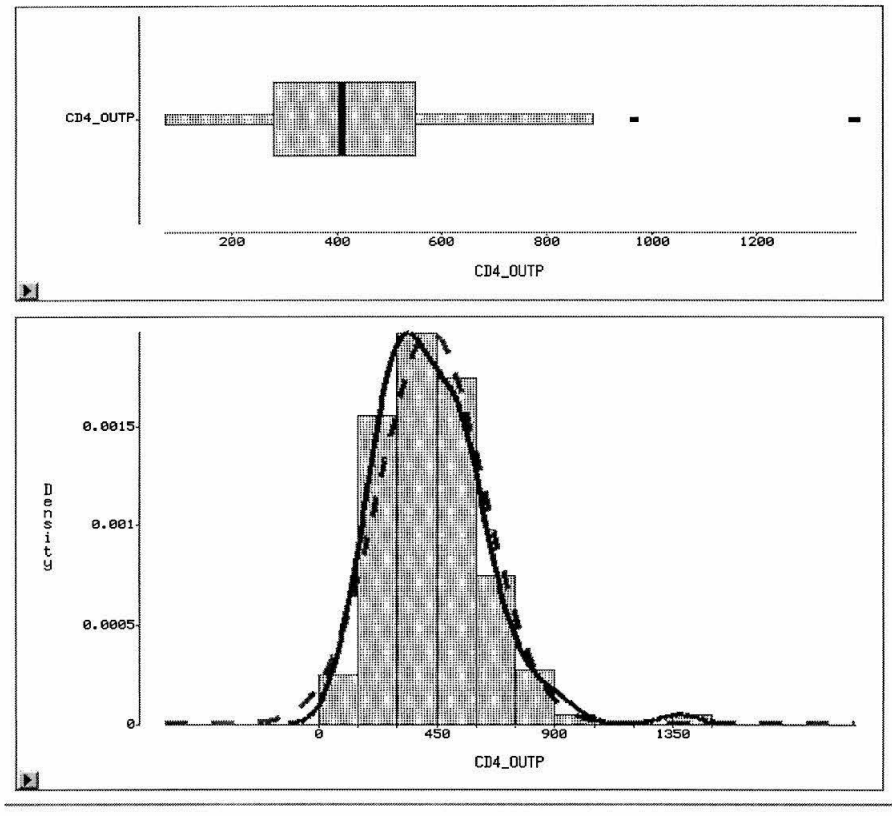

Figure 3.10: a. Box plot of CD4 T cell counts taken from the symptomatic stage of the outpatient dataset. b. Solid line: nonparametric density estimate of CD4 T cell counts taken from the symptomatic stage from the outpatient dataset. Broken line: data fitted to the Gaussian distribution.

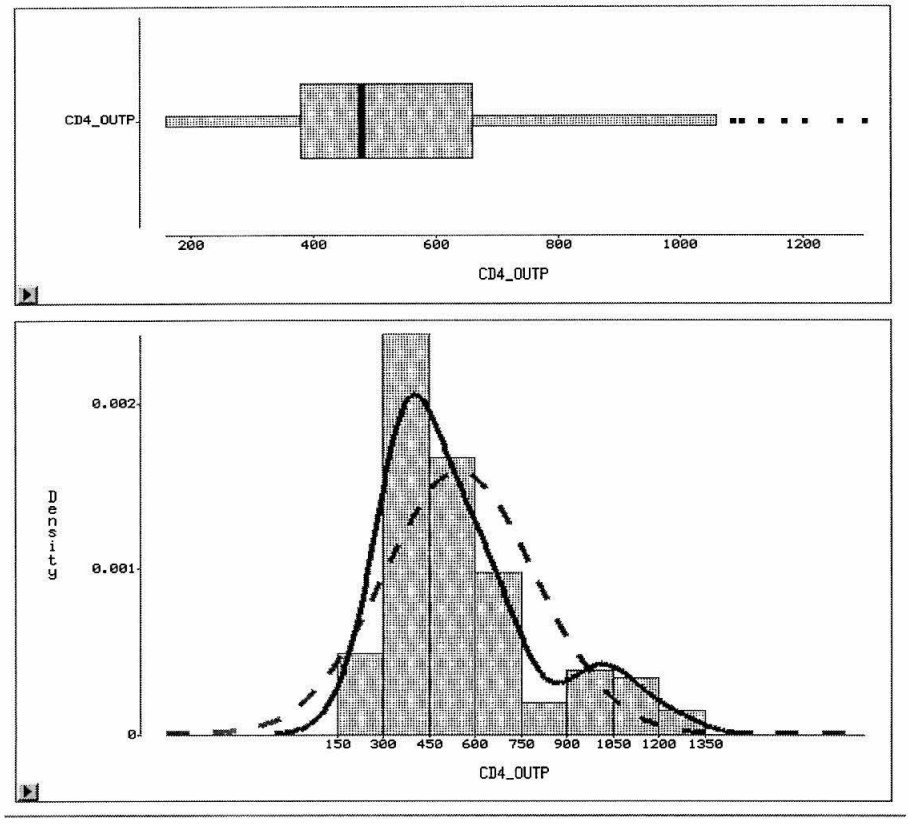

Figure 3.11: a. Box plot of CD4 T cell counts taken from the asymptomatic stage of the outpatient dataset. b. Solid line: nonparametric density estimate of CD4 T cell counts taken from the asymptomatic stage of the outpatient dataset. Broken line: data fitted to the Gaussian distribution.

Chapter 4 Nonparametric Density Estimation of Repeated Measures Data: An Application to CD4 T Cell Counts and Immune Reconstitution

Abstract

Analysis of longitudinal data, such as clinical markers like CD4+ T-lymphocyte (CD4) counts, are often complicated by the fact that there exists within-person correlation between the observations over time and there is often an unequal number of observations per person. Due to individual variation and differing responses, comparisons of means and variance of these levels may not be sufficient. In the specific case of CD4 counts, there might exist bimodality in the distribution, even when transformations such as the square root or log are performed. Assumptions of Gaussian distributions could lead to serious errors in cases of bimodality. Comparisons and analysis using means and standard errors could also be misleading. By employing a nonparametric density estimator, one can overcome these difficulties and compare distributions and levels. This paper outlines methodology to construct a nonparametric density estimator that is unbiased to order $O(h^2)$, consistent in mean square, and takes into account correlation and unequal number of observations. An application of the method is used to construct nonparametric density estimates of CD4 counts from both the Multicenter AIDS Cohort Study and a database of patients receiving Highly Active Anti Retroviral Treatment (HAART). It was found that the resulting distributions were distinctly non-Gaussian; therefore, use of traditional methods that assume underlying normality could yield misleading results. It was also found that the CD4 distribution in patients on HAART was significantly different from the CD4 distribution from the uninfected population. The results suggest that, on average, the CD4 levels of patients on HAART do not approach that of the uninfected population.

4.1 Introduction

CD4 T-lymphocyte (CD4) counts are important in monitoring and predicting the course of HIV infection. The onset of AIDS occurs when the level of CD4 counts is low enough to cause severe immunosuppression. Knowing the distribution of CD4 counts and the likelihood of AIDS is beneficial to implement effective prophalaxis for opportunistic infections. This is especially true when the prophalaxis can be toxic, invasive, or expensive. Repeated measures data are often complicated by correlation, and missing data. In examining CD4 counts, the analysis is also complicated by non-normality and in some cases bimodality. Estimation and comparisons of populations and levels are not straightforward in these cases.

It has been hypothesized that CD4 counts could be brought up to levels similar to the uninfected population if the virus could be suppressed long enough by combination antiretroviral therapy [11]. To test this, one could compare the distribution of CD4 counts from patients on combination antiretroviral therapy with the distribution of the uninfected population. The former distribution could be bimodal due to differing responses to treatment as well as genetic factors. In the clinical setting, often one set of patients responds favorably to treatment while other patients have a suboptimal response. The underlying distribution could be a mixture of several distributions. Looking at means and variances would be misleading in these cases. Thus comparisons to the uninfected population, whose distribution is reported to be Gaussian(1017,329) [28], could be misleading.

This paper outlines methodology for such a comparison and constructs a nonparametric density estimator that is unbiased and consistent in mean square. Because sequential observations of markers from the same individual are correlated [18], one cannot utilize the full data set and assume independence. It would also be inefficient to utilize only an independent subsample of the data [22]. The method described takes into account the within person correlation and is robust to unequal numbers of observations per person. The bootstrap is employed to estimate the standard errors.

This method is applicable to data from the Multicenter AIDS Cohort study

(MACS) and a database of patients receiving Highly Active Anti Retroviral Therapy (HAART). To demonstrate the inefficiency of subsampling, density estimates of CD4+ T-lymphocyte (CD4) counts were constructed utilizing the data from the MACS. A similar density estimation was constructed using an independent subset of the MACS. The independent distribution was not significantly different from the distribution that utilized the full sample and was less efficient. The patients were then segregated by AIDS status. Patients who had experienced an opportunistic infection were defined as having AIDS. It was found that the resulting distribution was bimodal, underscoring the importance of knowing the underlying distributional assumptions. The MACS distribution was then compared to the CD4 distribution constructed from the patients on HAART. The resulting distributions were compared and found to be significantly different. The distribution that was constructed from patients on HAART was compared to the hypothesized distribution of CD4 counts from the uninfected population. It was found that the CD4 distribution from the HAART patients was significantly different from the uninfected distribution, suggesting that patients in this sample did not experience a sustained CD4 T-lymphocyte cell increase great enough to be considered immune reconstitution. In the next section, the model is presented. The model is then applied to HIV data, and the results are presented.

4.2 Inference Procedures

Let X_{ij} be the jth observation on person i, $i = 1, ..., n; j = 1, ..., k$. Nonparametric estimates were constructed using

$$\hat{f}_n(x) = \frac{1}{nkh} \sum_{i=1}^{n} \sum_{j=1}^{k} K\left(\frac{x_{ij} - x}{h}\right) \tag{4.1}$$

where the kernel function, K, can be any probability density function.

Let K = the Normal kernel,

$$K(t) = \phi(t) = \frac{1}{\sqrt{2\pi}} exp\left(\frac{-t^2}{2}\right) \tag{4.2}$$

thus every data point receives Gaussian weight. The sum of these weights make up the CD4 density estimate,

$$\hat{f}_n(x,h) = \frac{1}{n-1}\sum_{i=1}^{n}\sum_{j=1}^{k}\frac{1}{\hat{\sigma}h}\phi\left(\frac{x_{ij}-x}{\hat{\sigma}h}\right), \tag{4.3}$$

where $\hat{\sigma}$ is the standard deviation of the $x'_{ij}s$.
Let the bandwidth, $h = (4/3)^{1/5}\hat{\sigma}n^{-1/5}$, where h is the Normal reference rule bandwidth for a Normal kernel [29].

The expectation of $\hat{f}(x)$ equals

$$E\hat{f}(x) = \int \frac{1}{h}K\left(\frac{x-s}{h}\right) f(s)ds = \int K(t)f(x-ht)dt \tag{4.4}$$

By Taylor expansion ...

$$E\hat{f}(x) = f(x)\int K(t) - hf'(x)\int tK(t) + \frac{1}{2}h^2 f''(x)\int t^2K(t) + ... \tag{4.5}$$

Since K equals the normal kernel, ϕ,

$$\int K(t) = 1, \int tK(t) = 0, and \int t^2K(t) \equiv \sigma^2 < \infty. \tag{4.6}$$

Therefore, $E\hat{f}(x) = f(x)$ to order $O(h^2)$, thus

$$Bias(x) = \frac{1}{2}\sigma^2h^2f''(x) = O(h^2) \tag{4.7}$$

In most longitudinal studies, observations between subjects can be considered independent. It is the within patient observations that are correlated. One can construct the underlying density of non-independent data, but one must take into

account the covariance terms when calculating the variance. The variance equals

$$Var(\hat{f}_n) = E\left(\frac{1}{n^2k^2h^2}\sum_{i=1}^{nk}\sum_{j=1}^{nk}\left[K\left(\frac{x_i - x}{h}\right) - h\bar{f}(x)\right]\left[K\left(\frac{x_j - x}{h}\right) - h\bar{f}(x)\right]\right) \tag{4.8}$$

where $f(\bar{x})$ is the expected value of $\hat{f}_n$. Let $z_i = K\left(\frac{x_i - x}{h}\right)$ and the variance can be written as

$$Var(\hat{f}_n(x)) = \frac{1}{(n^2k^2h^2)}\sum_{i=1}\sum_{j=1} Cov(z_i, z_j) \tag{4.9}$$

This can be extended to allow for k of different lengths for each person by letting $k = \max(k_i)$. Note that for each person, i, there exists at most k observations. Thus there will be at most nk^2 non zero covariance terms. Since $K = \phi, z_i$ and z_j are bounded. By the Cauchy-Schwartz inequality and the boundedness of K, there exists an $M < \infty$ such that $|\text{Cov}(z_i, z_j)| \leq M$;therefore:

$$Var(\hat{f}_n(x)) \leq \frac{1}{n^2k^2h^2}\sum_{i=1}^{nk}\sum_{j=1}^{k} \mid Cov(z_i, z_j) \mid < \frac{nk^2M}{n^2k^2h^2} = \frac{M}{nh^2} \to 0 \text{ as } n \to \infty \tag{4.10}$$

The k's drop out of the equation due to the fact that it was not assumed that correlations diminish over time. Furthermore, define $\sigma_{x,n} = \sqrt{\frac{M}{nh^2}}$ thus

$$\lim_{n\to\infty} n\sigma_{x,n} \to \infty \tag{4.11}$$

Therefore, by Scott [29], $\hat{f}$ is nonparametric and consistent in mean square. These results stem from the fact that there exists independence between the subjects. Thus only nk^2 terms are non-zero. Moreover, the fact that the k^2 term cancels ensures that the estimator is robust to unequal numbers of observations. Note also that h is constructed so that n goes to infinity faster than h goes to zero. Thus, $E(\hat{f}) \to f$ and $\hat{f} \to f$ as $n \to \infty$.

One could also subsample 1 observation from each person and get at most k iid

samples which would all be unbiased and consistent; but it would also be inefficient [22]. Estimating the variance empirically can be achieved through use of the bootstrap. Exploiting the fact that there exists between subject independence, one can bootstrap by rows and preserve the correlation structure. This is similar to the moving blocks bootstrap with block length k [30]. Let $i = 1, 2, ..., B$ denote the bootstrap samples, and let θ_i^* be the values of the statistic computed using each of these samples. The standard errors can be estimated by

$$\hat{se} = \left(\frac{1}{B-1} \sum_{i=1}^{B} (\theta_i^* - \bar{\theta}^*)^2 \right)^{\frac{1}{2}} \tag{4.12}$$

where

$$\bar{\theta}^* = \frac{1}{k} \sum_{i=1}^{B} \theta_i^* \tag{4.13}$$

The problem of edge effects come into play when the data are discontinuous or truncated. Boundary kernels, variable bandwidths, and reflective boundary techniques can be used to address these problems. Boundary kernels are sensitive to the choice of support intervals; thus one must be careful in the selection of the support interval. The kernel for a given point, x_i, covers the interval $(x_i - h, x_i + h)$. If the data are truncated at $x = 0$, the kernel interval should be $[0, x_i + h)$, which is narrower than $2h$. One solution is to use the wider interval $(0, 2h)$ for all $x_i \in [0, h)$ [31]. This is the variable bandwidth technique. The reflection boundary technique can be implemented if the discontinuity is at $x = 0$ and the data are nonnegative [29]. The technique is used on data that have been reflected about $x = 0$. Estimates are computed by taking the original estimates and doubling them for $x \geq 0$. The variable bandwidth technique is implemented on data in the AIDS stage, when CD4 counts approach zero.

4.3 Data

The methodology discussed in the previous section was applied to data from the Multicenter AIDS Cohort Study (MACS) public data set and to data from a private HIV clinic. The MACS is a prevalent cohort that collects data from volunteers at five separate centers. For each visit there was a physical examination, questionnaires and lab tests. The public data set starts at the beginning of the MACS in 1984 and ends October 1, 1992, and covers MACS visits 1-17. Subjects in this cohort were on therapies ranging from no treatment to combination nucleoside analogue therapy. The data end in 1992; consequentially, there are no subjects on protease inhibitors. The only selection criteria, for the data utilized in this analysis, was seropositivity. Observation of CD4+ T-lymphocyte counts on seropositive people yielded 2,166 patients with 19,998 person-visit observations[1]. Approximately 12.2 percent of these observations came from AIDS patients and 87.8 percent came from observations from subject who did not have an AIDS defining illness.

A separate data set, which was smaller but more detailed than the MACS, was collected from a private HIV practice. The data consist of a retrospective chart review of 213 patients who were on combination antiretroviral therapy (HAART). Data were gathered on clinical markers such as CD4+ T-lymphocyte counts and CD8+ T-lymphocyte counts. In the sample, 73 percent of the patients have private insurance, 16 percent are covered by government insurance such as Medi-care and Medi-Cal, and 11 percent of the patients have no insurance. This probably represents a higher percentage of privately insured patients and wealthier subjects than are representative in the general HIV population. This dataset is important because it offers more detail on the treatments that patients are taking. Approximately 33 percent of the observations came from the asymptomatic stage, 32 percent from the symptomatic stage and 35 percent of the observations came from patients who have had AIDS defining opportunistic infection.

The question of representativeness is intrinsic to disease cohorts [18]. This ques-

[1]Patients had 1 to 17 CD4 observations.

tion of selection bias is especially true of a single private clinic that services one geographic location. These patients are on and can afford the latest treatments and many are involved in clinical trials. The MACS data, on the other hand, was collected from five different centers and covered a wider spectrum of patients. Whether or not the data are representative is not the focus of this paper. The fact that private data has 35 percent of the observations coming from the AIDS population and 73 percent of the patients privately insured certainly is not representative of the entire population. We will use the methodology in the previous section to compare the CD4 distributions from the private dataset, the MACS dataset, and to the hypothesized uninfected distribution using a Kolmogorov-Smirnov test. Kolmogorov-Smirnov (KS) is appropriate for data of this type because it is distribution-free and searches over and compares the entire density. Nonparametric tests are preferable especially when there exists non-normality, skewness, and/or bimodality.

4.4 Analysis

Repeated CD4 measurements on the same person cannot be considered independent [18]. This suggests that a pooled sample of CD4 counts would have some degree of correlation. To ascertain the effects of the correlation on the distribution, an independent sample was drawn by randomly sampling one CD4 observation per person and estimating the nonparametric density. This sample is independent and the standard errors can be calculated in the usual fashion; it is, however, inefficient because it does not make use of all the data. It has also been shown that subsampling non-independent but stationary data can only result in poorer estimators [22, 23]. An independent subset of the MACS was created by taking a random subsample of one CD4 observation from each person. This resulted in a dataset of 2,166 independent observations. The nonparametric density distribution of the independent sample was estimated (Figure 1). The estimated mean was 474.56, the standard deviation was 306.02, and the standard error of the mean was 6.60. The estimation procedure was repeated using the full sample of 19,988 observations (see Figure 2). The mean was

483.27, and the standard deviation was 298.49. After 10,000 bootstrap replications the standard error of the mean was 5.70. The two distributions were then compared nonparametrically using the Kolmogorov-Smirnov (KS) test (see Table 1). The asymptotic KS statistic (KSa) was 1.35 and the prob > KSa was greater than 0.05. Therefore, the two distributions are not significantly different at the alpha equals 0.05 level. The nonparametric distribution was then plotted against the Gaussian curve. By inspection of the curves, one can see there exists a definite skew in the nonparametric distribution. A box plot of the data confirms this; see Figure 2. This suggests that the CD4 distribution of HIV infected patients may not be Gaussian. The distribution was then tested against the hypothesis of normality [24]. The hypothesis of normality was rejected $p < 0.001$ by KS. The Gaussian distribution does not present a good fit for the infected population due to the skewness and truncation at zero. Several other parametric distributions such as the log normal, exponential and Weibull were fit to the distribution with less success. One must take this result into account when comparing different populations of HIV infected individuals and comparing the HIV population to the uninfected population. The Kolmogorov-Smirnov test is completely nonparametric and searches the entire distribution, and thus appropriate for this type of situation. The square root transformation is applied to the CD4 counts, as standard in the literature [18, 25, 26, 27] and the CD4 density was reconstructed and given in Figure 3. The mean of this distribution is 20.74 with bootstrapped standard error 0.135. Using the Komologrov-Smirnov test for normality, the hypothesis that the estimated distribution is distributed Gaussian is rejected at p=0.01.

To further illustrate the necessity of testing the normality assumption when constructing estimates, the nonparametric density distribution of CD4 counts was constructed using only observations that came from patients with AIDS. The square root transformation is performed to normalize the data. The mean of this distribution is 13.62 with a bootstrapped standard error of the mean 0.164. Figure 4 shows that for the AIDS population from the MACS data, not only is the resulting distribution non-Gaussian (p=0.0001), it is bimodal. In the AIDS period, CD4 counts go to

zero, thus truncation at zero becomes a significant factor and techniques to account for edge effects must be used. Figure 5 shows the same distribution with a variable bandwidth for $x_i \in [0, h)$. Accounting for edge effects did not substantially change the distribution. The bimodality in the AIDS stage is important to consider when constructing hypothesis tests that rely on underlying normality. It is also important to consider when comparing means and variances of CD4 counts in the AIDS stage.

4.4.1 HAART Data

The nonparametric density of CD4 counts was estimated utilizing the entire sample from the private data. The mean for this distribution was 274.02 with standard error 195.00. The bootstrapped standard error of the mean was estimated to be 12.65 after 10,000 replications. The shape of the estimated distribution, shown in Figure 6, is skewed. The hypothesis of normality was rejected by KS, p=0.001. The MACS and the private data set distribution were compared by Kolmogorov-Smirnov and found to be significantly different at the p=0.001 level; see Table 2. The larger standard errors are more than likely due to the smaller sample size and shorter follow up period in the private data set. The difference in means can possibly be explained due to the fact that the MACS data set has only 12.2 percent of its observations coming from AIDS patients whereas the private dataset has approximately 35 percent of the observations coming from the AIDS stage. This could bias the mean downward.

The square root transformation was performed on the CD4 counts in attempt to normalize the data. The mean of the estimated distribution was 15.48 with bootstrapped standard error of the mean 0.385; see Figure 7. Normality is rejected by KS with p=0.0385. These results strongly suggest that the distribution of CD4 counts in HIV infected individuals are not distributed Gaussian.

To test the hypothesis of immune reconstitution in this population, the CD4 distribution was compared against the Gaussian(1017, 329) [28]. The hypothesis that the two distributions are similar was rejected by KS, p=0.0001. The same procedure was implemented to construct the distribution of CD8 counts. The mean of the

resulting distribution was 915.52 with bootstrapped standard error of the mean 12.65. The CD8 distribution from the MACS was compared to the CD8 distribution from the private data set. The estimated distributions were not significantly different at p=0.0918. However, when the CD8 distribution from the private data was compared to the Gaussian(614,234) [28], the distribution of CD8 counts from the uninfected population, they were statistically significant p=0.001 by KS. The results suggest that patients in this sample did not experience an increase in CD4 counts consistent with the hypothesis of immune reconstitution.

4.5 Discussion

A nonparametric density estimator was implemented on correlated data with unequal number of observations. Using this method, it was found that the estimated CD4 distributions from the MACS and from patients on HAART were distinctly non-Gaussian. Thus it is recommended that the assumption of normality, especially in the case of CD4 counts, be verified before parametric methods are used. If normality is not a valid assumption, then non-parametric techniques should be considered. The gain in efficiency from this estimator is not as great as one would hope. In the population that was large enough to check, there was a .9 decrease in the standard error of the mean. However, most samples are not large enough to warrant taking one observation from each person to obtain an independent sample. This is the real gain from this method. One can construct and compare estimates without the need for complete independence.

The results also suggest that, in this population, patients on combination anti retroviral therapy did not experience immune reconstitution. That is, their CD4 and CD8 levels did not increase to the levels of uninfected people. This discouraging fact might be due to patient failure to adhere to the drug regimen or HIV resistance.

4.6 Conclusion

This paper develops a methodology for comparing distributions where the observations making up the distributions are not fully independent, have unequal numbers of observations, and are distinctly non-normal. The method is nonparametric, unbiased to order $O(h^2)$, and consistent in the mean square. It is also easy to implement using GAUSS or other readily available statistical packages. Failure to consider these data limitations when constructing test statistics can yield misleading results. This paper also finds that patients on HAART did not experience immune reconstitution.

4.7 Figures

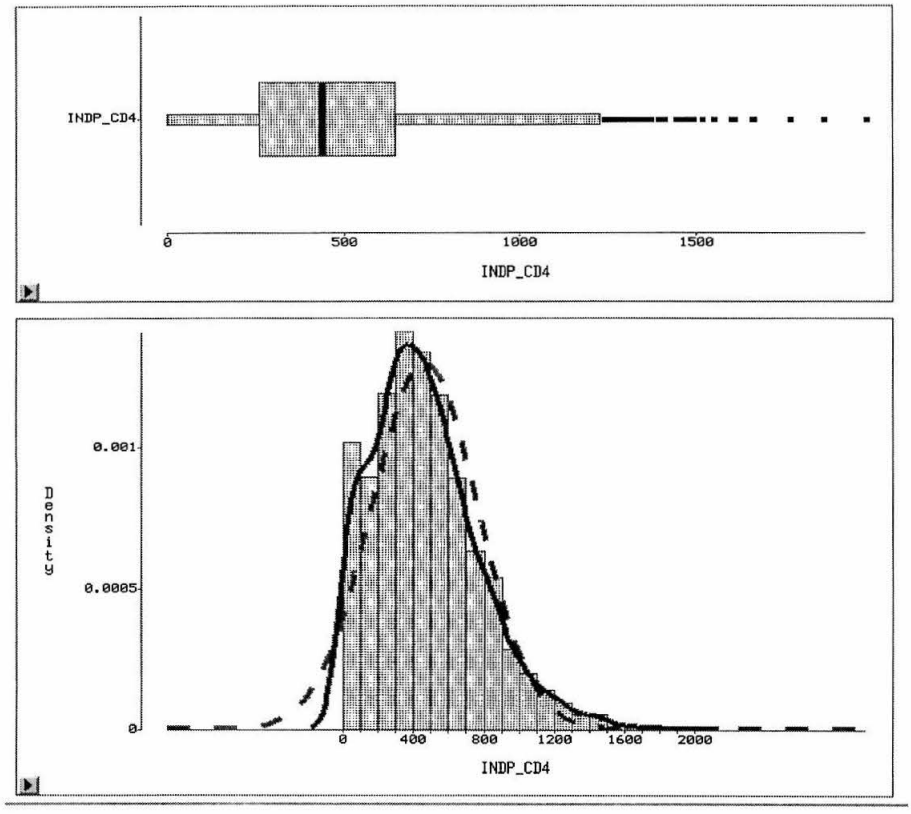

Figure 4.1: a. Box plot of 2,166 independent CD4 T cell draws from the MACS. b. Solid line: nonparametric density of CD4 T cell counts estimated from independent draws from the MACS. Broken line: data fitted to the Gaussian distribution.

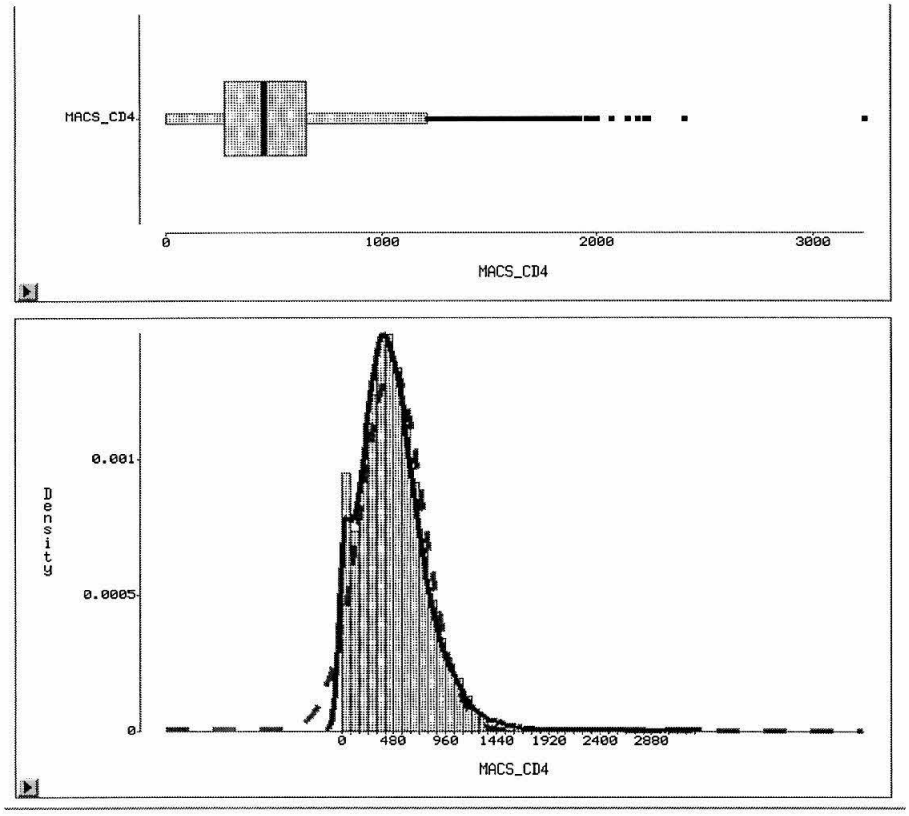

Figure 4.2: a. Box plot of 19,988 CD4 T cell observations from the MACS. b. Solid line: nonparametric density estimate of the CD4 T cell counts from the MACS. Broken line: data fitted to the Gaussian distribution

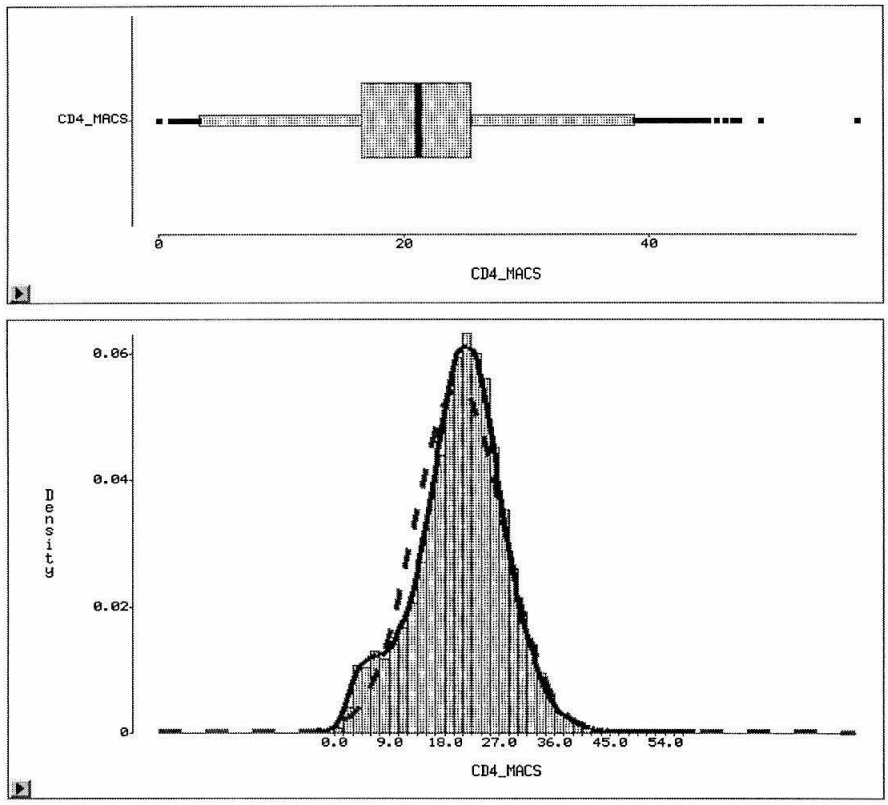

Figure 4.3: a. Box plot of square root CD4 T cell observations taken from the MACS. b. Solid line: nonparametric density estimate of CD4 T cell counts taken from the MACS. Broken line: data fitted to the Gaussian distribution.

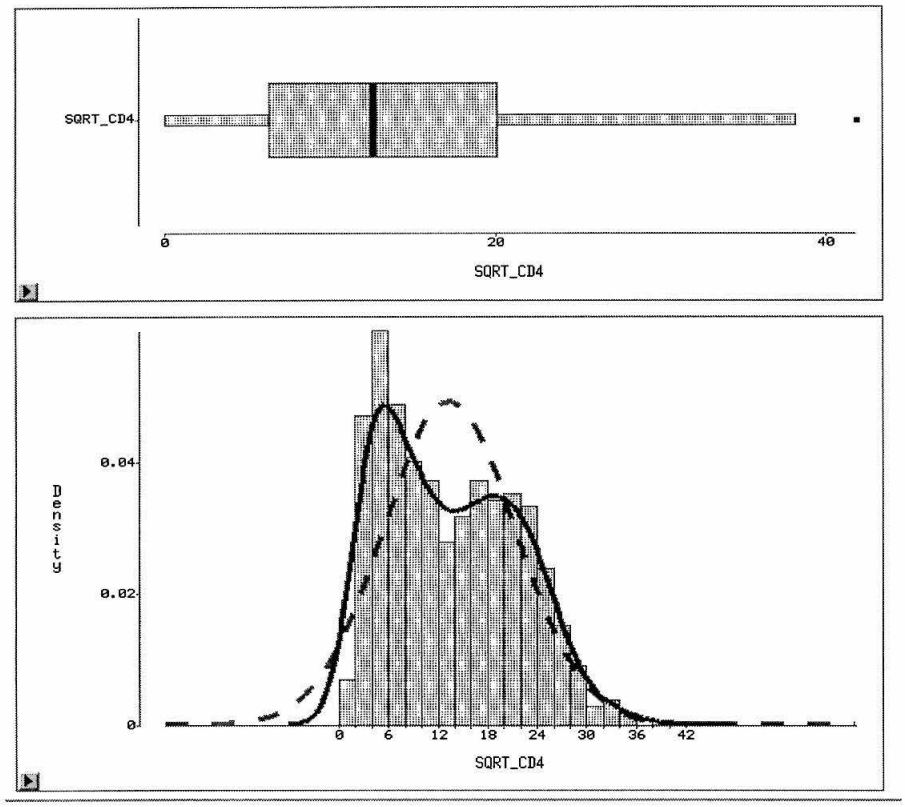

Figure 4.4: a. Box plot of square root CD4 T cell observations taken from the AIDS stage of the MACS. b. Solid line: nonparametric density estimate of CD4 T cell counts taken from the AIDS stage of the MACS. Broken line: data fitted to the Gaussian distribution.

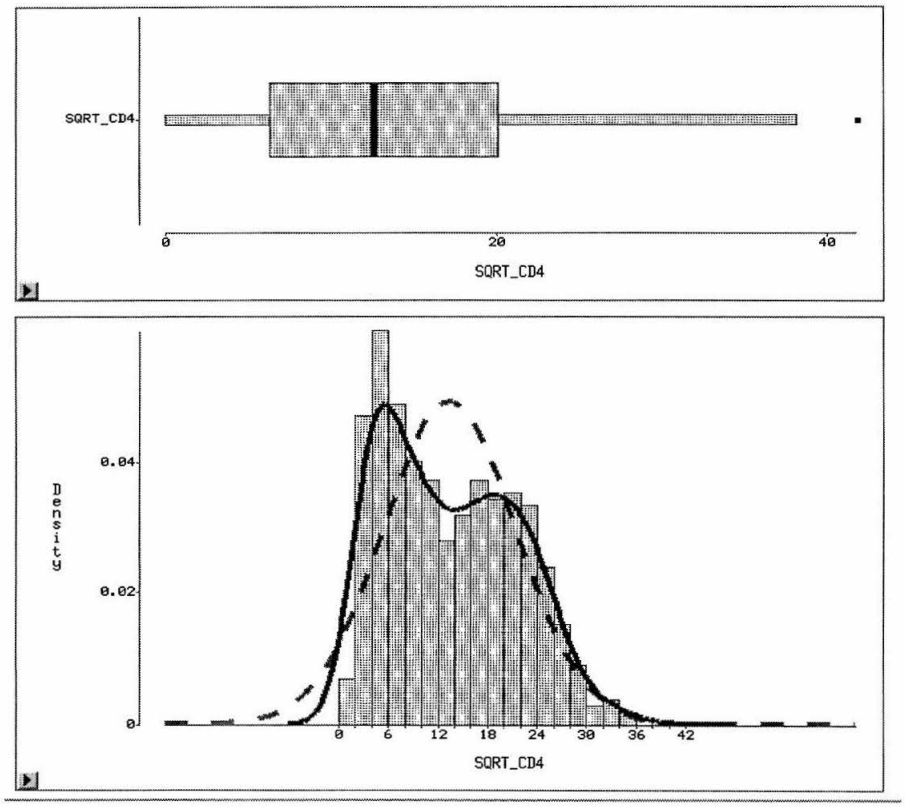

Figure 4.5: Nonparametric density estimate of square root CD4 T cell counts taken from patients with AIDS in the MACS and corrected for edge effects.

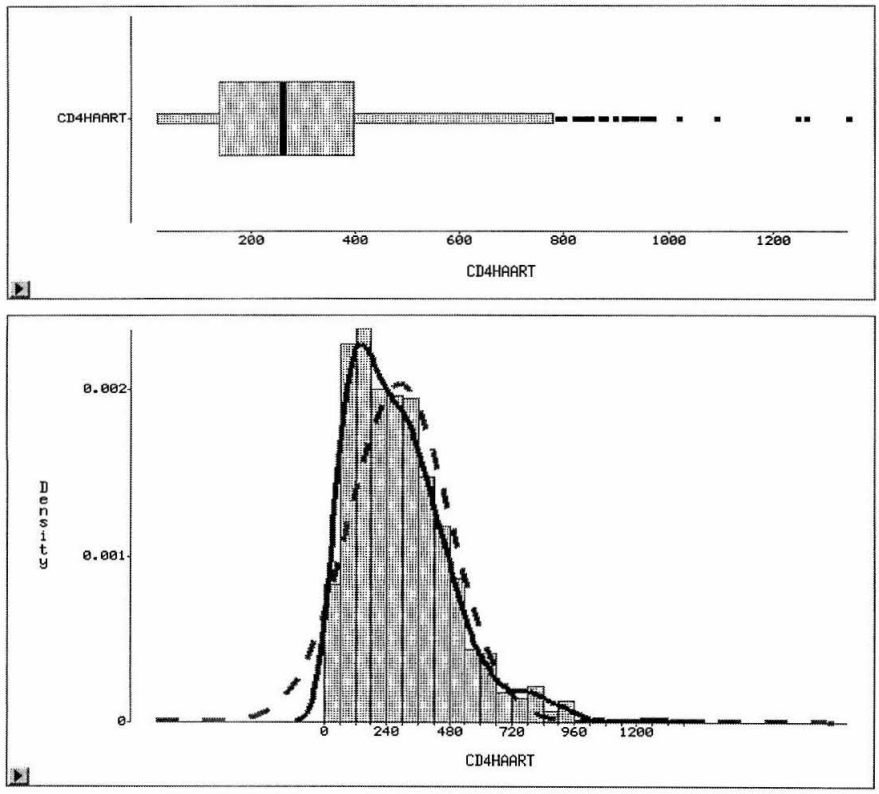

Figure 4.6: a. Box plot of square root CD4 T cell counts from the HAART dataset. b. Solid line: nonparametric density estimate of CD4 T cell counts taken from the HAART dataset. Broken line: data fitted to the Gaussian distribution.

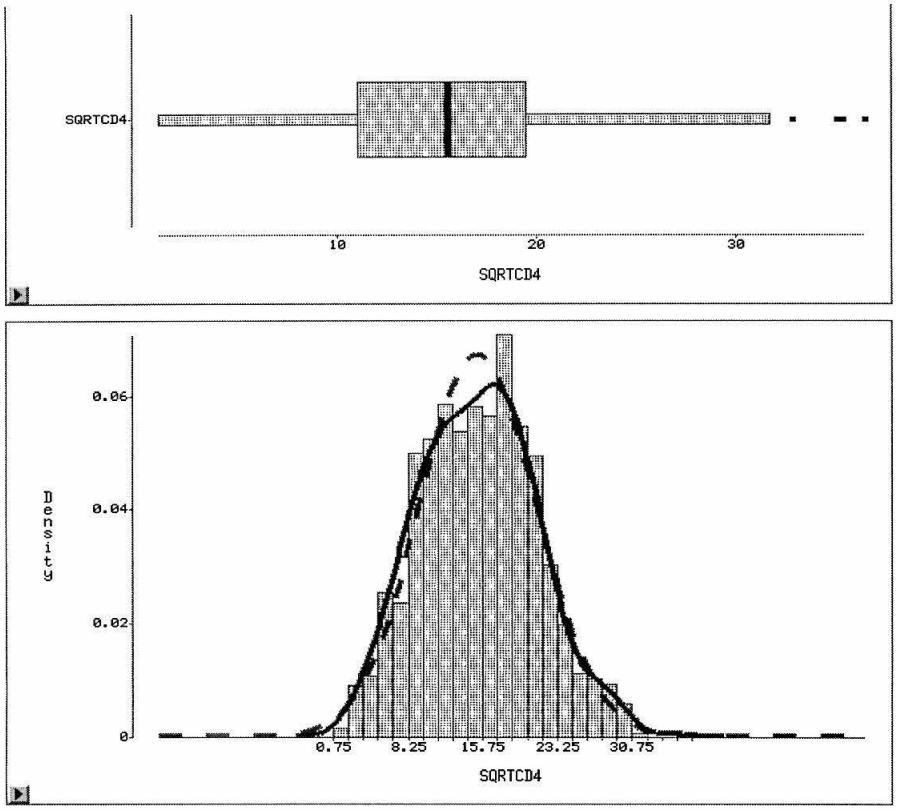

Figure 4.7: a. Box plot of square root CD4 T cell counts from the HAART dataset. b. Solid line: nonparametric density estimate of CD4 T cell counts from the HAART dataset. Broken line: data fitted to the Gaussian distribution.

4.8 Tables

	N	Mean	Standard Error of Mean
Independent MACS	2166	474.56	6.60
Full Sample MACS	19988	483.27	5.70
Asymptotic KS Statistic	1.33		p=0.08

Table 4.1: Independent MACS versus the full MACS

Sample	Mean	Standard Error	Standard Error of Mean (Bootstrapped)	p (versus Normal)
Full Sample (MACS)	483.27	298.48	5.70	0.001
Square Root CD4 (MACS)	20.74	7.27	0.0135	0.01
AIDS Observations (MACS)	13.62	3.20	0.1640	0.0001

Table 4.2: Testing the null hypothesis of normality

Dataset	N	Mean	Standard Error of Mean
HAART dataset	1534	274.25	12.65
MACS dataset	19988	483.27	5.70
KS test of equality of distributions			p=0.001

Table 4.3: Testing the equality of distributions from the MACS and HAART data

	Mean	Standard Error
HAART dataset	274.25	195.01
Uninfected population	1017	329.00
KS test of equality of distributions		p=0.0001

Table 4.4: Testing the equality of distributions from uninfected people and the patients in the HAART dataset

Sample	Mean	Standard Error	Standard Error of Mean (Bootstrapped)	p (versus Normal)
CD4 (HAART dataset)	274.25	195	12.62	0.001
Square root CD4 (HAART)	15.475	5.93	0.3800	0.0385

Table 4.5: Testing the null hypothesis of normality for the HAART dataset

Chapter 5 Clinical Failure of Highly Active Antiretroviral Therapy in HIV-1 Infected Patients

Abstract

Background: Dramatic reductions in morbidity and mortality from HIV/AIDS have been attributed to the introduction of highly active antiretroviral therapy (HAART). To assess the long-term effects of HAART, we retrospectively examined 213 patients on long-term HAART.

Methods: 213 HIV-1 infected patients from an outpatient clinic were retrospectively evaluated after a median of 30 months on HAART. Patients entered the study when first placed on HAART and were followed-up until May 1998. The primary endpoint was diagnosis with an AIDS defining condition.

Results: Median time until clinical failure for patients on long-term HAART was 585 days. In multivariate proportional hazards analyses for subjects with baseline CD4 T-cell counts of <100, 100-199, 200-400, and 400+ cells/mm^3, the relative hazard (RH) of progression was 9.992, 4.221, and 2.991 respectively ($p<0.01$). Baseline viral load was not significant. Patients with greater initial responses to HAART had decreased risk of progression, RH=0.0722 p=0.0016. Increases in viral load increased risk of progression, RH=1.421, p=0.0001. Increasing CD4 cell counts over time reduced the risk of progression, RH=0.934, p=0.010. Patients diagnosed with an opportunistic infection (OI) prior to initiation of HAART were five times more likely to progress than other patients, RH=5.415 p=0.001. Median time until clinical failure was 399 days for patients with a previous OI. Patients naive to antiretrovirals had reduced risk of progression compared with those pretreated, RH=0.417 (p=0.039).

Conclusions: Despite initial decreases in HIV RNA levels, an unexpectedly high level of clinical failure was found in patients on long-term HAART with a median failure time of 585 days.

Keywords: HAART, clinical failure, disease progression, HIV

5.1 Introduction

The use of highly active antiretroviral therapy (HAART) to treat HIV disease using combinations of reverse transcriptase and protease inhibitors has led to dramatically reduced morbidity and prolonged life in HIV-1 infected patients [10, 32] The initiation of HAART rapidly and significantly reduces the levels of virus replication in the peripheral blood [33, 34, 35]. In the HIV-infected patient, higher baseline levels of HIV RNA and lower baseline levels of CD4+ T lymphocyte cells in the peripheral blood are strongly predictive indicators of disease progression [36, 25]. However, the prognostic values of HIV-1 RNA levels and CD4+ T-cell counts are poorly defined in patients on long-term HAART and for patients with more advanced disease progression, such as those with AIDS defining illnesses and severely depleted CD4+ T-cells. As the treatment of HIV infected patients using HAART is relatively recent, little is known about long term effectiveness.

Unfortunately, in the clinical setting, many therapeutic regimens fail within weeks to months after initiation, prompting the administration of salvage therapy with a different combination of drugs [37, 38] These regimens are intended to prevent the emergence of drug resistant viral isolates. Many salvage therapeutic regimens ultimately fail, however, due to cross-resistance of viral isolates. Moreover, it may not be possible to find a combination of drugs to which there would not be pre-existing resistance.

Long-term effectiveness of HAART is poorly understood. The objectives of this study were to delineate the factors associated with disease progression in an unselected cohort of HIV-1 infected patients on long term HAART. Survival analysis was performed to assess the clinical failure rate of initial HAART and subsequent salvage regimens, as determined by diagnosis with an AIDS defining condition, and to assess the median time until clinical failure. The parameters surrounding clinical failure were assessed to yield insight as to the long-term effectiveness of HAART and its potential to control HIV-disease progression.

5.2 Methods

5.2.1 Study Subjects

The factors that have previously been associated with disease progression in HIV in large cohorts have been baseline quantitative HIV-1 RNA levels [36, 39] and baseline CD4 T cell count [25, 40]. These studies indicate that HIV-1 RNA levels (viral load) are strongly predictive of HIV disease progression. However, the relative prognostic value of CD4 T cell count and viral load remains poorly defined in cohorts on long-term HAART and for patients with advanced disease. Most of the studies to date have focused on patients who had high CD4 T cell counts and were relatively asymptomatic [36, 41]. In the clinical setting, physicians treat patients in all stages of the disease.

The data presented here are from an unselected cohort of outpatients. We retrospectively analyzed 213 patients from clinical chart data collected from December 1996 until May 1998. All patients were on individualized treatment regimens and were on HAART continuously for a median of 30 months. HAART, in this study, is defined as a combination of three or more antiretrovirals where at least one is a protease inhibitor. In most of the patients it was necessary to administer a salvage regimen consisting of different combinations of antiretrovirals due to intolerance or rising plasma HIV-1 RNA levels.

The study population consisted of 195 men and 18 women. The median age at baseline was 41 years. Plasma HIV-1 RNA levels and CD4 T-cell counts were performed, on average, every other month. Median HIV-1 RNA at baseline was 4.239 log copies/ml (range, 1-5.8 log copies/ml). Median baseline CD4 cell count was 230 cells/mm^3 (range, 0-1011 cells/ml). Thirty-seven (17 percent) patients were antiretroviral naive before starting HAART and 32 (15 percent) had experience with three or more reverse transcriptase inhibitors before starting HAART. Opportunistic infections were diagnosed in 51 patients before the start of HAART. Table 1 lists the characteristics of patients at baseline.

5.2.2 Statistical Analysis

Clinical failure was defined as diagnosis with an AIDS defining opportunistic infection (OI) or CD4 T cell counts falling below 200 cells/mm^3. For patients who were diagnosed with an opportunistic infection prior to the start of the HAART regimen (N=51), clinical failure was defined as the time until the next opportunistic infection. A separate analysis was conducted with clinical failure defined as a diagnosis with an opportunistic infection only, regardless of CD4 T cell count, and yielded similar results (results not shown). Parameters associated with clinical failure were analyzed. The parameters included age, baseline CD4+ T cell count, baseline viral load, viral load over time, CD4 T cell count over time, initial virologic response to HAART, and history of opportunistic infections. In addition, pretreatment with antiretrovirals prior to the start of HAART was also considered.

Data were analyzed using SAS version 6.11 (SAS Institute, Cary, NC). Survival time was measured as the date of therapy start until an AIDS defining event or censoring time. Failure time was calculated by the Kaplan-Meier method. Comparisons of survival across subgroups were performed using the log-rank test and the Cox proportional hazards model adjusted for left censoring to allow for staggered entry into the study. Associations among subgroups were examined with Kaplan-Meier plots and proportional hazards models, including partial likelihood ratio Chi square statistics. All p-values are two-sided.

5.3 Results

The characteristics of the 213 subjects are given in Table 1. To determine the time until clinical failure, survival analyses using Cox's proportional hazards model and Kaplan Meier were performed. Of the 213 subjects, 94 progressed to an AIDS defining endpoint. Figure 1 shows the Kaplan-Meier estimates of the proportion of subjects who did not experience clinical failure. The median time until clinical failure was 585 days (95 percent confidence interval (CI): 505-647 days); see Table 2.

In the outpatient clinic, patients enter treatment at different stages of the disease. To test whether prior diagnosis with an opportunistic infection (OI) was a significant factor in clinical failure, both univariate and multivariate proportional hazards models were estimated. It was found that patients diagnosed with a prior opportunistic infection (OI) were more than five times more likely to experience clinical failure than patients who did not have an OI prior to starting HAART, RH=5.415 (p=0.0001). The patients were then stratified by opportunistic infection status prior to the start of HAART and Kaplan Meier curves were estimated. Patients with a previous history of an OI (N=51) had a median of 399 days until the onset of another OI; see Table 3. Patients who were not diagnosed with an opportunistic infection prior to the start of HAART had a median of 623 days until clinical failure; see Table 4. The difference between the two groups is significant at p=0.0001. Kaplan-Meier curves for patients with an OI prior to HAART and those without an OI prior to HAART are given in Figure 2.

To assess the impact of antiretroviral pre-treatment before HAART, patients were stratified according to their previous experience with antiretrovirals. Thirty-seven antiretroviral naive patients were compared against 146 pre-treated patients. The relative risk for patients who were naive was significantly less than for patients who were pretreated, RH=0.417 (p=0.039). HAART offers the best chance of long-term viral suppression for patients who start HAART antiretroviral naive. This result suggests that drug resistance may be playing a significant role in clinical failure of HAART.

It has been shown that CD4+ T-cell counts are an important predictor of disease progression [36, 25, 40]. For purposes of analysis of CD4 T cell counts and the relationship to clinical failure, it was necessary to redefine clinical failure as an opportunistic infection only. For this part of the analysis only, CD4 T count below 200 cells/mm^3 was not considered an AIDS defining event. In both univariate and multivariate analyses, baseline CD4 T cell counts was an important predictor of disease progression for this patient group. Patients with higher baseline CD4 T cell counts had a decreased risk of progression, RH=0.884 (p=0.001). Baseline CD4 T cell counts were

then stratified into clinically useful categories (less than 100, 100-199, 200-400, and greater than 400 cells/mm^3). Kaplan Meier estimates of the proportion of patients who did not experience clinical failure were constructed for each stratum and given in Figure 3. Cox proportional hazards model was utilized to estimate the relative hazard of progression based on baseline CD4 T cell count. Subjects with CD4 T cell counts less than 100 cells/mm^3 were nearly ten times more likely to progress than subjects whose CD4 T cell counts were above 400 cells/mm^3 (RH=9.992, p=0.0001). Subjects with CD4 T cell counts between 100-199 cells/mm^3 were more than four times and subjects with CD4 counts between 200 and 400 cells/mm^3 were three times more likely to progress to AIDS, RH=4.221 (p=0.001) and RH=2.991 (p=0.01) respectively. These results suggest that baseline CD4 T cell counts have a significant effect on disease progression for patients on HAART and patients with higher baseline CD4 T cell counts have a decreased risk of clinical failure. The fact that patients are experiencing clinical failure despite HAART suggests that the increases in CD4 T cell counts due to HAART may be insufficient to prevent clinical failure.

Other studies have found that baseline viral load is a significant predictor of disease progression [39, 36, 25]. In this study baseline viral load and age were not significant in predicting clinical failure, p>0.25. HAART has a dramatic effect on both HIV-1 RNA levels (viral load) and CD4 T cell counts over time. We tested whether changes in these measures over time were significant. Changes in the levels of viral load and CD4 T cell counts, constructed as changes of the most recent test result from baseline, were very predictive. Increases in viral load from baseline were associated with increased risk of progression, RH=1.421 (p=0.0001); see Table 5. Changes in CD4 T cell counts from baseline that resulted in an increase were associated with a decrease in the risk of progression, RH=0.934 (p=0.01).

Since HAART has the most rapid and dramatic effect on viral load, we tested whether the initial virological response was predictive of clinical failure. Virologic response was measured as the initial decrease in HIV-1 RNA levels from baseline after HAART implementation. It was found subjects with more dramatic responses (greater changes in HIV RNA levels after HAART initiation) had a decreased risk

of progression than those who had a more modest response, RH=0.722 (p=0.0016). Therefore, for each 1.0 log copies/ml decrease in HIV-1 RNA levels after HAART initiation, the hazard of clinical failure decreased by an estimated 27.8 percent.

5.4 Discussion

The use of HAART to treat HIV-infected patients has led to dramatically decreased morbidity and mortality. However, the failure of HAART to eradicate HIV [14, 15] leaves the patient vulnerable to the emergence of drug-resistant virus and resurgence of viral replication [12]. Wong et al. [12] found that the persistence of even low levels of detectable virus in the peripheral blood due to incomplete viral suppression reflects ongoing replication in the lymphoid system and the emergence of drug resistance. We performed a retrospective study to assess the clinical failure rate and the factors associated with the risk of disease progression in a cohort of patients undergoing long-term HAART in an outpatient clinic. A high rate of clinical failure was found, with the median failure rate of 585 days (95 percent confidence interval: 505-647 days) after the initiation of HAART. Patients who experienced an opportunistic infection before HAART implementation (N=51) had an increased risk of disease progression and a lower median time until clinical failure. This suggests that the initiation of antiretroviral treatment prior to rather than following the onset of an opportunistic infection is more effective in preventing or slowing down disease progression. Furthermore, patients who were naive to antiretroviral treatment prior to the start of HAART (N=37) had a decreased risk of clinical failure compared to patients who were antiretroviral experienced (p=0.039). It was found that patients with a greater initial virologic response to HAART, measured as initial decrease from baseline viral load at HAART implementation, had a decreased risk of progression compared to patients with a more modest response (p=0.0016). For each 1.0 log copies/ml decrease in HIV-1 RNA levels after therapy start, the hazard of clinical failure decreased by an estimated 27.8 percent. It was also found that patients with higher baseline CD4 T cell counts had a decreased risk of clinical failure compared to patients with lower baseline CD4 T

cell counts. Thus, these results suggest that HAART should be implemented early in HIV disease and that primary attempts with antiretroviral therapy offer the best chance for long-term viral suppression and increased time until AIDS.

Our results indicate a high rate of clinical failure in an unselected cohort in the outpatient setting. Almost all of the patients experienced a rebound in viral load during administration of HAART at which time salvage therapy regimens that included different combinations of antiretrovirals to which the patient was naive. Despite continued efforts to control virus replication through the introduction of different combinations of drugs, most patients experienced clinical failure by progressing to an AIDS defining event. This disappointing result reflects the persistence of virus in infected patients and may be caused by the emergence of drug resistant virus, breakthrough of viral replication, or patient non-compliance to the treatment regimen.

Patient noncompliance is an increasingly important issue when discussing long-term treatment in an outpatient clinic. Patients who missed doses were not dropped from the study as long as the patient never fully stopped all antiretroviral medications. This intent-to-treat analysis yields results that mirrors more what is seen in the clinic as opposed to what is seen in the more controlled environment of a clinical trial. Levels of noncompliance, determined by the physician questioning the patient if they had taken all of their medications at each visit, were measured around 30 percent. Recent analysis has shown that noncompliance is a significant factor for clinical failure ($p=0.01$).

Although many patients experienced clinical failure, only six died. This possibly suggests that HAART might have some therapeutic benefit that is not reflected in CD4 T-cell counts or HIV-1 RNA levels. This could also be a function of effective prophalaxis and treatment medication for opportunistic infections. Further follow up is necessary to test whether the decreases in mortality due to AIDS will continue to decline. However, the results suggest that the incidence of AIDS will increase as more and more patients continue to fail their HAART regimens. It seems that HAART was not "hard enough" [11]. Even for patients who are less experienced, the fact that HAART does not completely eradicate the virus leaves them susceptible to viral

mutation and breakthrough. New classes of drugs with different resistance profiles as well as strategies to boost the immune system are needed. Moreover, these results underscore the importance of the vaccine effort.

5.5 Figures

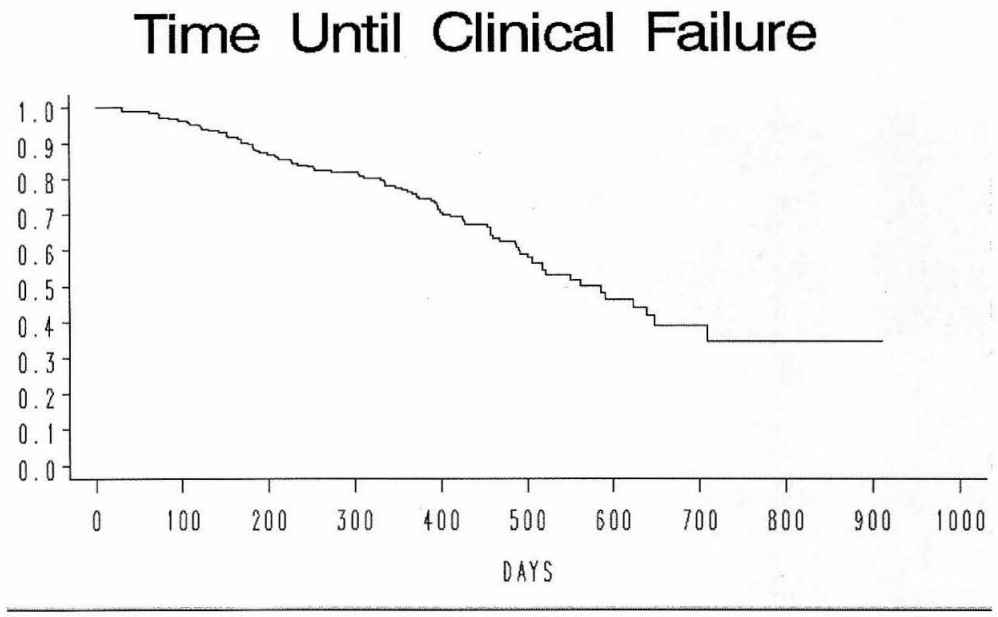

Figure 5.1: Kaplan-Meier estimates of the proportion of subjects in whom the primary endpoint of clinical failure was not reached. The y axis shows the proportion of patients left in the sample who have not experienced clinical failure. The x axis is time on HAART in days.

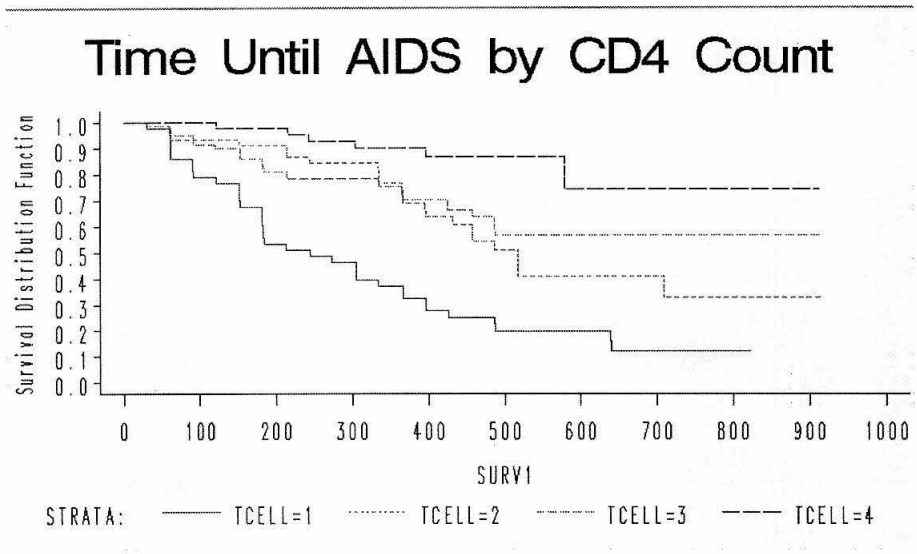

Figure 5.2: Kaplan-Meier estimates of the proportion of subjects in whom the primary endpoint of clinical failure was not reached stratified by baseline CD4 T cell counts. The y axis shows the proportion of patients left in the sample who have not experienced clinical failure. The x axis is time on HAART in days. The black line represents the survival curve of patients with a baseline CD4 count of less than 100 cells/mm^3. The red line represents the survival curve of patients with 100-199 cells/mm^3. The green line shows the survival curve for patients with 200-399 cells/mm^3. The blue line shows the survival curve of patients with 400+ cells/mm^3. P<0.001 for the comparison between subjects with greater than 400 cells/mm^3 and subjects with less than 100 cells/mm^3 and 100-199 cells/mm^3. P=0.01 for the comparison of subjects with greater than 400 cells/mm^3 and subjects with 200-399 cells/mm^3.

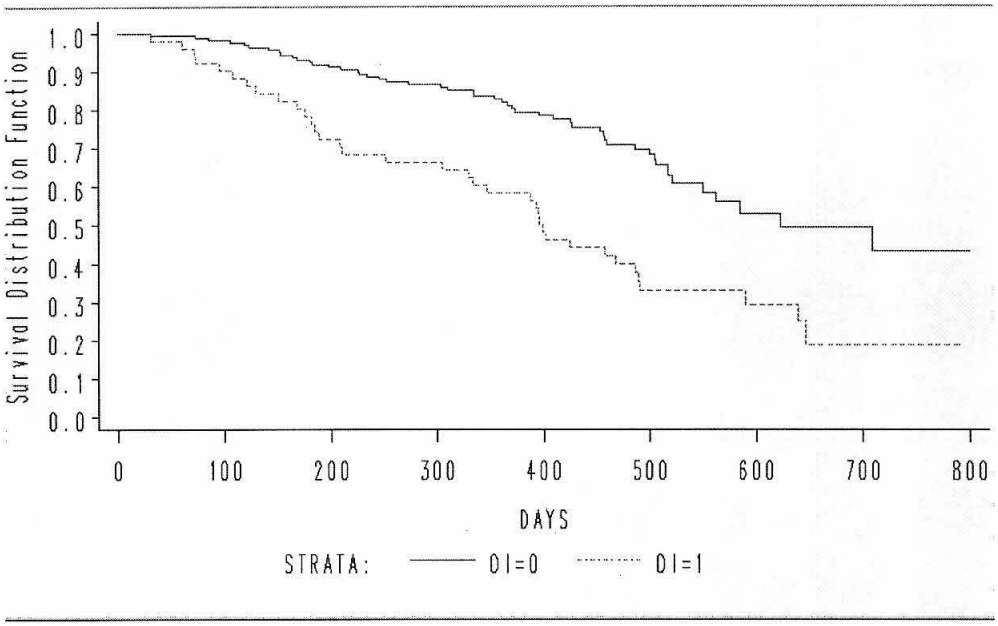

Figure 5.3: Kaplan-Meier estimates of the proportion of subjects in whom the primary endpoint of clinical failure was not reached. The y axis shows the proportion of patients left in the sample who have not experienced clinical failure. The x axis is time on HAART in days. The solid line represents patients who did not have an opportunistic infection (OI) prior to the start of HAART. The dashed line represents patients who were diagnosed with an opportunistic infection before HAART was started. P=0.0001 for the comparison between subjects who were diagnosed with an OI prior to HAART and subjects who did not experience an OI prior to the start of HAART.

5.6 Tables

Table 1. Characteristics of the 213 Patients at Base Line

Sex - no. (%)	
Male	195 (92%)
Female	18 (8%)
Age in years - median (range)	41 (21-69)
Clinical stage	
Asymptomatic (CDC stage A)	86 (40%)
Symptomatic (CDC stage B)	76 (36%)
Clinical AIDS (CDC stage C)	51 (24%)
CD4+ T cell count -cells/mm^3	
Median (range)	230 (0-1011)
0-99 cells/mm^3 - no. (%)	43 (20%)
100-199 cells/mm^3 - no. (%)	45 (22%)
200-399 cells/mm^3 - no. (%)	80 (36%)
400+ cells/mm^3 - no. (%)	45 (22%)
Log HIV RNA -median copies/ml (range)	4.239 (1-5.8)
Antiretroviral experience	
Antiretroviral naïve - no. (%)	37 (17%)
Moderate - < 3 drugs - no. (%)	114 (68%)
Heavy - 3+ drugs -no. (%)	32 (15%)

Table 5.1: Characteristics of the 213 patients at baseline

Table 2. Summary Statistics for Time until AIDS (in days)

Quantile	Point Estimate	95% Confidence Interval	
75%	----	709	----
50%	585	505	647
25%	371	310	426
Mean 513.27	Standard Error 16.04		

--- refers to insufficeint data to estimate

Table 5.2: Summary statistics for time until AIDS in days

Table 3. Summary Statistics for Patients with previous OI (in days)

Quantile	Point Estimate	95% Confidence Interval	
75%	639	486	590
50%	399	330	490
25%	185	130	334
Mean 394.54	Standard Error 29.93		

Table 5.3: Summary statistics for patients who experienced an opportunistic infection prior to the initiation of a HAART regimen

Table 4. Summary Statistics for Patients without Previous OI (in days)

Quantile	Point Estimate	95% Confidence Interval	
75%	nd	nd	nd
50%	623	550	nd
25%	454	366	506
Mean 552.99	Standard Error 17.94		

nd refers to insufficeint data to calculate

Table 5.4: Summary statistics for patients who did not experience an opportunistic infection prior to the start of HAART

Table 5. Analysis of Maximum Likelihood Estimates

Variable	Parameter Estimates	Standard Error	Wald Chi Square	Pr > Chi Square	Risk Ratio
Age	-0.0167	0.0136	1.464	0.2264	0.984
Change CD4	-0.0677	0.0287	5.574	0.0128	0.934
Change HIV RNA	0.3510	0.0755	21.642	0.0001	1.421

Table 5.5: Analysis of Maximum Likelihood Estimates

Chapter 6 Limited Viral Suppression with Salvage Therapy After Combination Antiretroviral Therapy Failure in HIV-1 Infected Patients

Abstract

We retrospectively evaluated 67 HIV-1 infected patients with clinical anti-retroviral resistance to assess the effectiveness of salvage therapy. Clinical resistance was defined by fixed-elevated or rising plasma RNA PCR levels. All patients were failing combination anti-retroviral regimens which included at least one of the following protease inhibitors: saquinavir, indinavir, or ritonavir. Salvage therapy regimens that included the protease inhibitor nelfinavir plus two to four other antiretroviral medications were instituted. Viral load decreased on average by 0.55 $\log_{10}$ copies/ml and the decrease was sustained on average for five months before returning to baseline levels. Average CD4 counts rose by an average of 43 cells/mm^3 however; this was not statistically significant. The median time until virologic failure was 87 days, with 91.1 percent of the population failing by 225 days. The "hit early, hit hard" [11] approach may work well with those newly infected or treatment naive. However, these patients constitute a minority in most HIV practices. For patients who are experienced with several antiretroviral agents or for whom response to primary treatment was sub-optimal, options for future treatment may be limited due to the presence of resistance-conferring mutations. Our results confirm that salvage therapy, even when there is at least one novel agent, is not effective in producing a durable virologic response in heavily treated patient populations.

Keywords: Salvage therapy-HIV infection-Combination therapy failure-Drug resistant HIV-1.

6.1 Introduction

A growing number of patients with HIV-1 infection have fixed-elevated or rising plasma viral load levels despite highly-active anti-retroviral therapy (HAART) [42, 43, 27, 44, 45]. In this circumstance, treatment regimens are usually modified (salvage therapy) in an effort to achieve better suppression of plasma viral load [46, 47]. Sequential monotherapy with nucleoside analogue reverse transcriptase (RT) inhibitors in the era prior to the introduction of protease inhibitors (PI) is recognized as one major cause of failure of combination RT-PI regimens; another is poor patient adherence to these regimens [42, 27]. Both allow the evolution of resistance mutations which may diminish the effectiveness of salvage regimens [48, 12, 49]. It has been reported that in as many as 50 percent of cases, anti-retroviral therapy failed to control viremia [50]. Prior failure of antiretroviral agents and cross-resistance can result in few viable option for subsequent treatment [51, 52, 53].

We retrospectively evaluated 67 patients who had clinical resistance to combination anti-retroviral therapy that included at least one of the other FDA-approved protease inhibitors (indinavir, saquinavir, or ritonavir). At the time of salvage therapy implementation, all of the patients were nelfinavir naive. Preliminary data suggested that nelfinavir might have a distinct resistance pattern that might not overlap with other protease inhibitors [54, 55]. Salvage regimens were selected individuals to ensure a combination of antiretrovirals to which the patients were naive. In some cases, this was not possible so viral genotypic testing was performed to help guide the selection of salvage drugs for these patients. All salvage regimens included nelfinavir plus two to four other antiretrovirals were initiated. After 86 days, roughly half of the population experienced virologic failure. Virologic failure is defined resurgence of HIV RNA in plasma. After 225 days, 91 percent of the population had failed. At the end of the follow up (approximately 640 days), 6/67 (8.9 percent) patients had undetectable viral loads. Overall, for the overwhelming majority of patients (61/67) we did not see a sustainable virologic response to salvage therapy after primary attempts of combination therapy failed.

6.2 Methods

6.2.1 Study Population

The data were constructed from a retrospective chart review of 67 HIV-1 infected patients in a primary HIV care office, who were failing combination anti-retroviral therapy as indicated by fixed-elevated or rising plasma RNA viral load. Subjects gave consent under the condition that their anonymity be preserved. All subjects were nelfinavir naive, and initially received nelfinavir through an expanded access program. There were 60 men and 7 women. The average age was 43. The median viral load was 3.52 $\log_{10}$. The median $CD4^+$T-lymphocyte count (CD4 count) was 254 cells/mm^3 and the median $CD8^+$T-lymphocyte count was 973 cells/mm^3. Fourteen patients had a prior AIDS defining illness. See Table 1. Most of the patients in our study were heavily pre-treated. Table 2 shows for each patient, antiretrovirals that were taken prior to the start of the salvage therapy regimen. 96 percent had prior AZT experience, 94 percent had prior 3TC experience, and 82 percent had prior indinavir experience. All patients were protease experienced. 19.4 percent were experienced with all 3 FDA approved protease inhibitors at the time (indinavir, saquinavir, and ritonavir). Viral genotypic testing [1] was performed on 23 patients prior to the initiation of the salvage regimen. For the patients who were genotyped, antiretroviral drug histories were particularly complex or unreliable. The results of the genotypic testing were used to help guide the choice of salvage regimen.

Individual salvage regimens were selected by review of patient's medication histories, histories of adverse effects, and for those who were genotyped, the resistance information [56, 57]. This was done to ensure that patients were given combinations of antiretrovirals to which the virus would be the most susceptible. Since all patients were naive to nelfinavir, it was chosen as one of the protease inhibitors for the regimens. In 28 out of 67 patients, nelfinavir was used in combination with another protease inhibitor. The most common salvage therapy regimens were: D4T, 3TC, nelfinavir (18), two nucleoside analogues (AZT/3TC, D4T/3TC, or D4T/DDI) plus

[1] Genotypic analysis was performed at Specialty Labs in Santa Monica, CA.

nelfinavir and saquinavir (12), AZT, 3TC, nelfinavir (11), two nucleoside analogues plus nelfinavir and nevirapine (9), two nucleoside analogs plus nelfinavir, saquinavir, and nevirapine (8), and one nucleoside analogue plus nelfinavir and saquinavir (6). The most common previous therapies that patients were failing at the time of the switch were: AZT, 3TC, indinavir (29 patients), D4T, 3TC, indinavir (15), and AZT, 3TC, saquinavir, ritonavir (17). Follow-up time was approximately six months.

6.2.2 Statistical Methods

Virologic failure was defined viral resurgence. Data were analyzed using SAS version 6.11 (SAS Institute, Cary, NC). Survival time was measured as date of start of salvage regimen until virologic failure. Failure time was calculated by the Kaplan-Meier method. A repeated measures analysis of variance with a teoplitz covariance matrix was used to analyze the different time points of viral load and CD4. Details of this procedure are in the appendix.

6.3 Results

The median time to virologic failure was 86 days. After 225 days roughly 91 percent of the patients experienced failure. See Figure 1 and Table 3. This dramatic failure rate underscores that fact primary attempts of combination therapy offer the best chance of controlling viral replication. Secondary, or salvage attempts, were not, for the vast majority of the population, able to produce a sustainable virologic response.

The mean viral load in the baseline regimen was 3.52 $\log_{10}$. After two months, viral load was 2.97 $\log_{10}$; this represents a 0.55 log decrease in viral load. The decrease was significant at the p=0.047 level. At four months, viral load was 2.95 $\log_{10}$. By 6 months viral load 3.16 $\log_{10}$. This was not significantly different from the baseline viral load (p=.1445). See Table 4. The plot of mean viral loads over time are presented in Figure 2.

In the analysis of CD4 counts, we took the square root of CD4 counts to stabilize the variance [26]. The mean and standard errors of the square root of CD4 counts

are presented in Table 5. The numbers presented in the text have been transformed back to original counts. The mean CD4 count at baseline was 217 cells/mm^3 (14.71 on the square root scale). After two months of salvage therapy, the average count rose to 244.0 cells/mm^3 (15.6 on the square root scale). At the four month period average CD4 counts were 259.2 cells/mm^3 (16.1 square root scale). By six months, CD4 counts were 257.3 cells/mm^3 (16.04 square root scale). The differences in CD4 counts at all time periods were not significantly different from baseline CD4 ($p > 0.26$). Figure 3 shows the plot of CD4 counts over time on the square root scale.

Only six patients (8.9 percent) had an undetectable viral load[2] at 450 days, i.e., achieved the goal of HAART [58]. The overwhelming majority of patients, 61, had an average decrease of 0.48 log which returned to baseline by six months. To better understand this result, we stratified the sample by indinavir failures (n=55) and ritonavir or saquinavir failures (indinavir naive)(n=12). We also examined whether the inclusion of the non-nucleoside reverse transcriptase inhibitor (nnrti), nevirapine, in those patients who were naive to this class of agents made a significant difference in the virologic response compared to the salvage regimens that did not contain an nnrti. Patients who had failed ritonavir or saquinavir and were indinavir naive had a 1.27 log copies/ml drop from baseline viral load after initiating the salvage regimen (p=0.001). After six months viral load levels returned to near baseline levels (p=0.14). Two patients in this group had undetectable viral loads at the end of six months. The indinavir experienced group had a marginal decrease (.27 log copies/ml) after salvage therapy implementation (p=0.04) and returned to baseline by six months (p=0.72).

We also examined the subset of patients who received nevirapine as part of their salvage regimen. This group had a 0.94 log copies/ml drop from baseline viral load after initiating salvage therapy (p=.0015). This decrease was sustained until month 6 when it was not significantly different from baseline (p=0.61). Two patients in this group had an undetectable viral load at the end of the study. Patient regimens that did not include nevirapine had a .45 log drop in viral load from baseline (p=.0051). Month six viral load was not significantly different from baseline (p=0.4159).

[2]The lower limit of detection is 25 copies/ml.

Viral genotyping for HIV anti-retroviral drug-resistance was performed in 23 patients prior to the change in regimen. Genotypic analysis was performed only for patients with particularly complex drug histories. For these patients we found that 91 percent of the viral isolates had the 215 mutation in the reverse transcriptase gene, which is the primary mutation for AZT [59, 60]. The 184 mutation, the principal mutation for conferring 3TC resistance [61, 62, 63], was present in 73 percent of the isolates. In the patient isolates, 42 percent had mutations at codons 184, 210, and 215 which correlate with *in vitro* resistance to AZT and 3TC. Fewer than 10 percent of patient isolates had mutations at codons 50 or 75, which is associate *in vitro* resistance to D4T. In the protease gene, 68 percent of patient isolates had mutations at codon 90 which is a principal mutation for *in vitro* saquinavir resistance [64]. Mutations at codon 82, a principal mutation for both indinavir and ritonavir [65, 66], was found in 36 percent of patient isolates. Mutation at codon 84, associated with multiple protease resistance [67, 44, 68, 52], was found in 36 percent of the isolates. Figures 4 and 5 show the percentage of the patient isolates with each mutation in both the reverse transcriptase gene and the protease gene. On average, patients in this sub-population had 7 mutations in the reverse transcriptase gene and 4 mutations in the protease gene.

A second ANOVA analysis was performed on the genotypic information to see which mutations were predictive of changes in $\log_{10}$ viral load. Patient isolates with mutations concurrently at protease codon 90 and RT codons 184, 210, and 215 had significantly higher $\log_{10}$ viral load at month six, $p = 0.04$. Patient isolates with mutations occurring simultaneously at protease codon 82, and RT codons 184, 210, and 215 had a significantly higher $\log_{10}$ viral load at month 6, $p = 0.04$. Patient isolates with mutations at both protease codons 82 and 84 had a higher viral load at month 6 than those who did not have these mutations, $p = 0.009$.

6.4 Discussion

Overall we did not observe a sustained virologic response to salvage therapy in our patient group. Within 87 days, approximately half the population experienced virologic failure and within 225 days 91 percent had failed. Viral load remained undetectable in only 6/67 (8.1 percent) of patients. These patients had, on average, higher baseline CD4 counts and lower baseline viral loads than the rest of the population. See Table 6. Suggesting that early intervention for salvage regimens might be beneficial.

A sustained decrease in viral load greater than 0.5 log is generally considered biologically significant [69]. In our patient population, the decrease in average viral load was not sustained beyond six months, thus it is not clear whether or not these salvage regimens had any longterm effects on survival or quality of life. In other studies, decreases in viral load correlate with a significant benefit in survival. O'Brien [25] found that each threefold (0.5 log) decrease in viral load was associated with a 63 percent reduction in the relative hazard of progression ($p = 0.02$). Even more modest decreases of 0.3 log, if sustained, were associated with a 27 percent reduction in the relative hazard of progression [70]. The observed increase in CD4 counts in our patients might be expected to contribute some clinical benefit, but the level of increase was marginal, especially when compared to other studies such as the Swiss Cohort [27].

We believe that the failure to achieve sustained viral suppression in our population relates to extensive pre-treatment and to a high prevalence of resistance mutations among patient isolates. Indeed, the seven patients whose viral load remained undetectable at six months were the least pre-treated. Almost half of the patient isolates that were genotyped had mutations associated with resistance to AZT and 3TC and greater than 36 percent of the isolates were cross-resistant to multiple protease inhibitors (due to the 82 and 84 mutation). This helps to explain the relatively modest effects of the salvage regimens on viral load.

Winters et al. [49] recently reported that resistance patterns for saquinavir and nelfinavir overlap significantly. They reported that isolates that had reduced sus-

ceptibilities *in vitro* to nelfinavir had either the 48 or 90 mutations. In our study, 89 percent of the patient isolates had either the 48 or the 90 mutation. Thus prior protease inhibitor failure likely contributed to a suboptimal response to nelfinavir in the salvage combination regimens. Suboptimal response to nucleoside analogue RT inhibitors no doubt occurred as a result of prior combination therapy or serial monotherapy. This reinforces the fact that primary attempts at combination therapy have the most potential to induce remission of viremia. Secondary efforts (i.e., salvage therapy) will inevitably be at a disadvantage until new agents with non-overlapping resistance patterns become available.

Plasma HIV RNA levels as well as CD4 counts are primary indicators that guide therapy modification. A patient's drug treatment history should be the major factor that directs the choice of a salvage regimen. Genotypic and phenotypic resistance testing may also be useful to help guide the choice of alternate salvage regimens for individual patient management [56]. For our patients, drug treatment history was the primary tool used to select the salvage regimen. For patients with complex treatment histories, genotypic testing was performed to help determine a feasible salvage regimen. Nelfinavir was chosen because it was a novel protease inhibitor whose preliminary resistance profile appeared promising for salvage therapy in that the pattern of resistance was different from other protease inhibitors [54, 55]. This, however, was not the case as was later demonstrated by Winters et al. [49] *in vitro* and confirmed by ours and others clinical experience.

Genotypic and phenotypic assays may prove useful in selection of the most effective combination and sequences of antiretroviral agents [56]. Treatment with drugs that have differing resistance patterns would delay the emergence of cross-resistant viral strains [64]. Reserving agents for later use may preserve future treatment options. In retrospect, sequential treatment had a deleterious effect on the rate of virologic remission and limited the option of the patients. Our experience reinforces the need for caution and careful planning in the timing and selection of salvage regimens. A sustained result with nelfinavir containing regimens was precluded in part because of an unforeseen overlap in the resistance profile, prior protease inhibitor failure, and

lack of drugs in other classes to which the patients were naive. Utilization of nnrti's in the 17 patients who were nnrti-naive in all likelihood precludes further use of these agents in combination. This experience illustrates the dilemma faced by clinicians in utilizing antiretrovirals that are introduced sequentially.

6.5 Appendix

The statistical method we use tests whether the decrease in viral load and the increase in CD4 counts were significant. In our statistical model we control for effects due to time, therapy, and the interaction of therapy over time using fixed effect parameters. Our model specifies a random effect for individuals in different treatment groups to allow for the fact that individuals may have different responses to treatments. Because repeated observations such as viral load and CD4 counts in individuals tend to be correlated, we could not assume independence between the observations. We chose a more general framework for our variance-covariance matrix that estimated this covariance and incorporated it into the model. Failure to take this correlation into account can lead to biased standard errors and misleading results. We constructed a more complicated model to correctly estimate the standard errors.

The model we specify is a two-factor analysis of covariance that distinguishes treatment and time:

$$y_{ijk} = \alpha_j + \beta_k + \pi_{ij} + \alpha\beta_{jk} + \varepsilon_{ijk}, \tag{6.1}$$

where y_{ijk} is the $\log_{10}$ viral load (or square root CD4 count) for person i on therapy j at time k. α_j is a fixed effect for the therapy regimen $j = 1, 2$. β_k is a fixed effect for time period $k = 0, \ldots, 3$. In our study, $j = 1$ for the baseline regimen and $j = 2$ for the salvage therapy regimen. Time period, $k = 0$ denotes the baseline while $k = 1, 2, 3$ denotes follow up periods measured at 2 month increments: 1 month, 3 months, and 5 months.

Allowing for the fact that individuals have differing responses to different treat-

ments, we let π_{ij} be a random effect for the *ith* patient on treatment j. We assume that the π_{ij} are independent normally distributed $N(0, \sigma^2)$. The $\alpha\beta_{jk}$ term is a fixed effect for the interaction of therapy and time. We specify that ε_{ijk} is a random error whose elements are not required to be independent. We assume that π_{ij} and ε_{ijk} are uncorrelated. We also assume (ε_{ijk}) has expectation 0 and denote its variance-covariance matrix by R. Since the correlation of viral loads over time for a given individual is not constant, we used a general covariance matrix which allows for unequal correlations over time. The covariance matrix for individual i, R_i, has Toeplitz structure with:

$$R_i = \begin{pmatrix} \sigma^2 & \sigma_1 & \sigma_2 & \sigma_3 \\ \sigma_1 & \sigma^2 & \sigma_1 & \sigma_2 \\ \sigma_2 & \sigma_1 & \sigma^2 & \sigma_1 \\ \sigma_3 & \sigma_2 & \sigma_1 & \sigma^2 \end{pmatrix},$$

where σ_i is the correlation of the baseline observation with the *ith* observation. Thus R will be an MxM[3] block diagonal matrix.

Following Lindstrom and Bates [71] and Jennrich and Schluchter [72], equation (1) may be written:

$$y = X\beta + Zv + \varepsilon, \tag{6.2}$$

where y is a matrix of log viral loads or square root CD4 counts. Continuing, X is a matrix of indicator variables for the fixed effects and Z is a matrix of indicator variables for the random effects. β is a vector of unknown parameters and v is a random vector whose distribution is assumed multivariate normal with expectation zero and variance-covariance matrix G; i.e., $v \sim N(0, G)$. Following the assumptions above, $G = \sigma^2 I$. Therefore, the covariance matrix of y, Σ, can be written as:

$$\Sigma = R + ZGZ'. \tag{6.3}$$

[3]In a balanced sample $M = NxK$, where N is the number of patients in the sample (67) and K is the number of time periods (4).

It follows that $y \sim N(X\beta, \Sigma)$. Since G equals $\sigma^2 I$, clearly $ZGZ' = \sigma^2 I \otimes 11'$. Thus Σ, like R, will be block diagonal. It will also be symmetric about the diagonal and positive definite.

Estimation of this model was performed by maximum likelihood using a ridge-stabilized Newton-Raphson algorithm [73, 74] in SAS. We obtained estimates of the mean and standard errors of log viral loads and square root CD4 counts for all patients in the study population in the baseline, and the three subsequent follow-up periods. The standard errors from the least squares means were computed by the method of Henderson [75]. To test the specification of our model after it was estimated, we performed a likelihood ratio test. The likelihood ratio test compares our mixed model covariance structure to a null model, where the null model is the standard linear model containing just the fixed effects and a simple residual variance of $\sigma^2 I$. The likelihood ratio test rejected the null model, ($p = 0.0001$), in favor of our specification.

6.6 Figures

Figure 1 – Kaplan Meier curves of time until failure in days

Figure 2 – Mean Log Viral Load Over Time

Figure 3 – Mean Square Root CD4 Counts Over Time

Figure 4 – Percentage of Population with Reverse Transcriptase Mutations

Figure 5 – Percentage of the Population with Protease Mutations

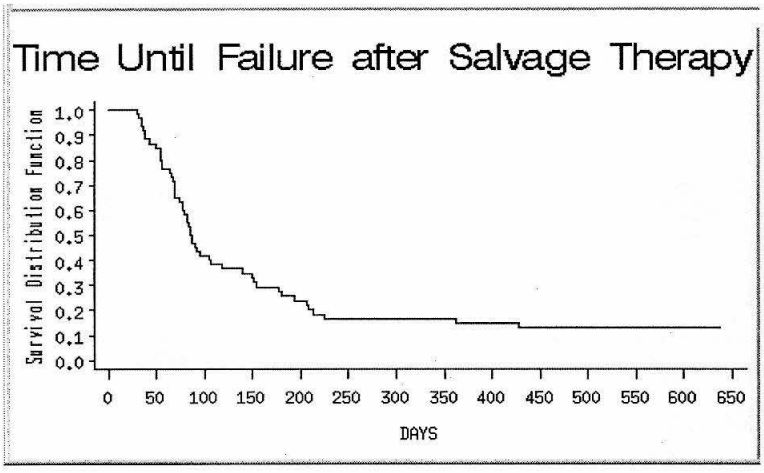

Figure 6.1: Time until virologic failure after implementation of salvage therapy. The y axis shows the proportion of patients who have not failed. The x axis shows days until failure.

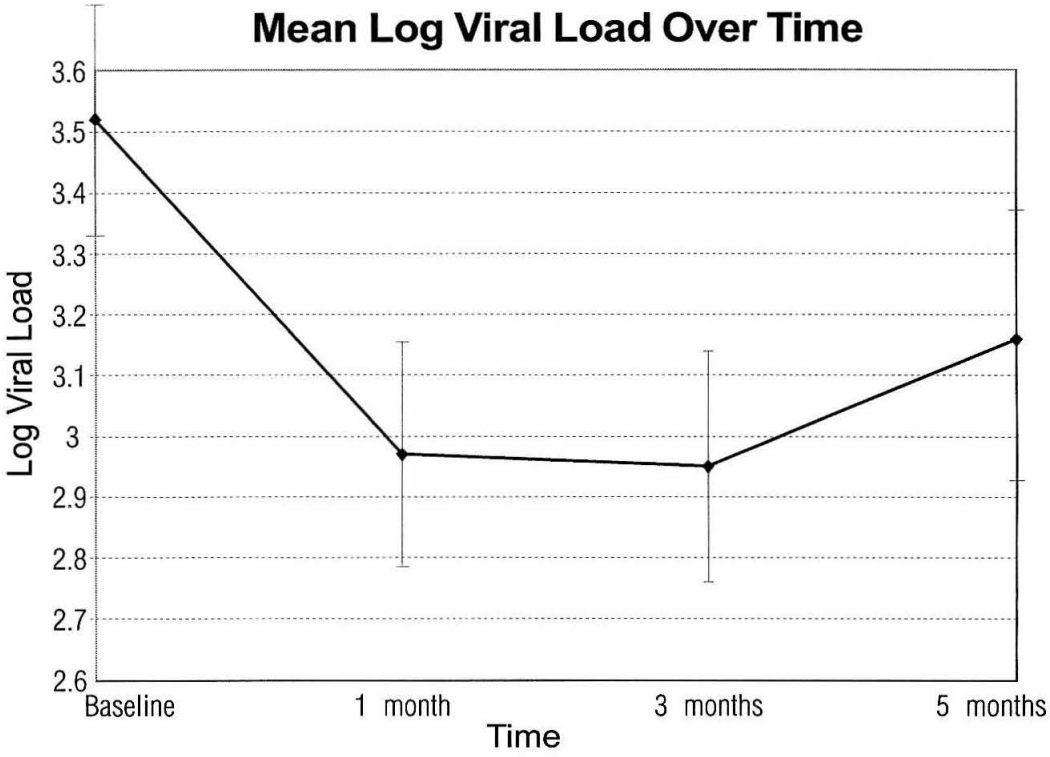

Figure 6.2: Mean log viral load over time.

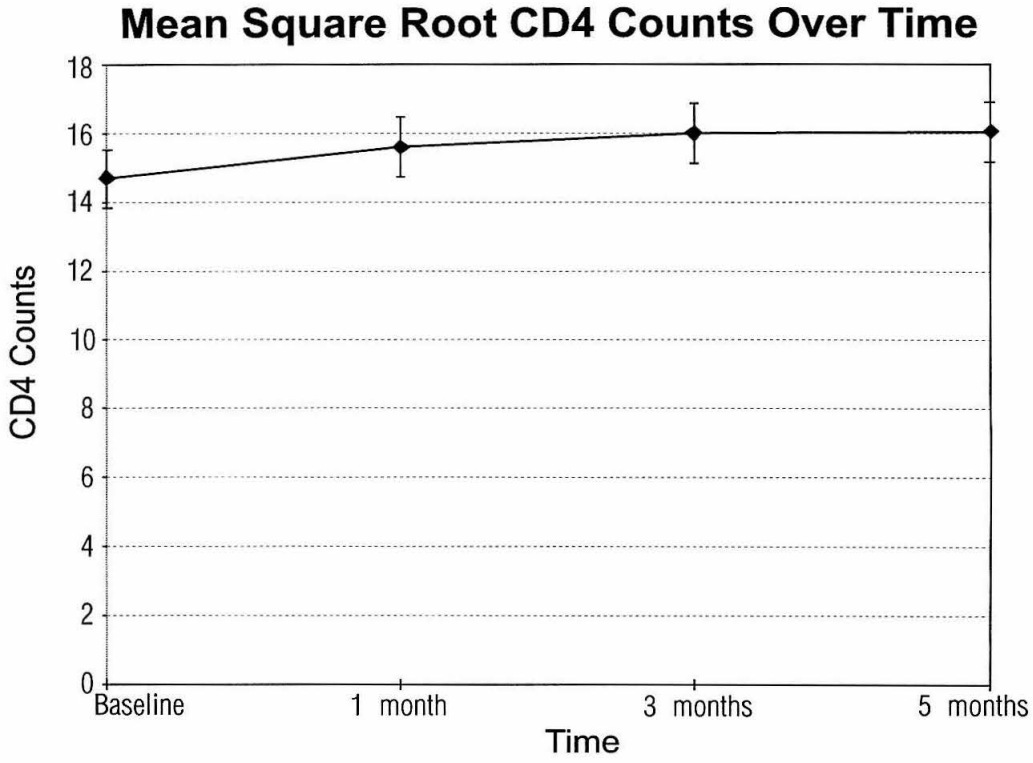

Figure 6.3: Mean square root CD4 T cell counts over time.

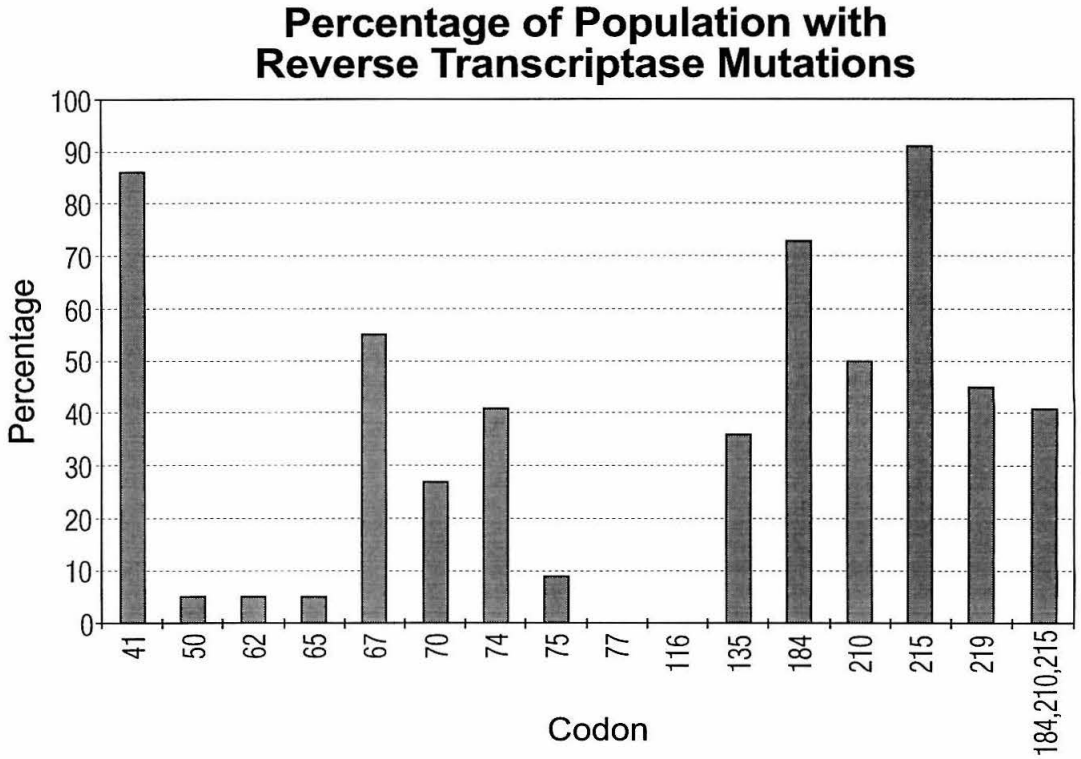

Figure 6.4: Percentage of the population with specified reverse transcriptase mutations.

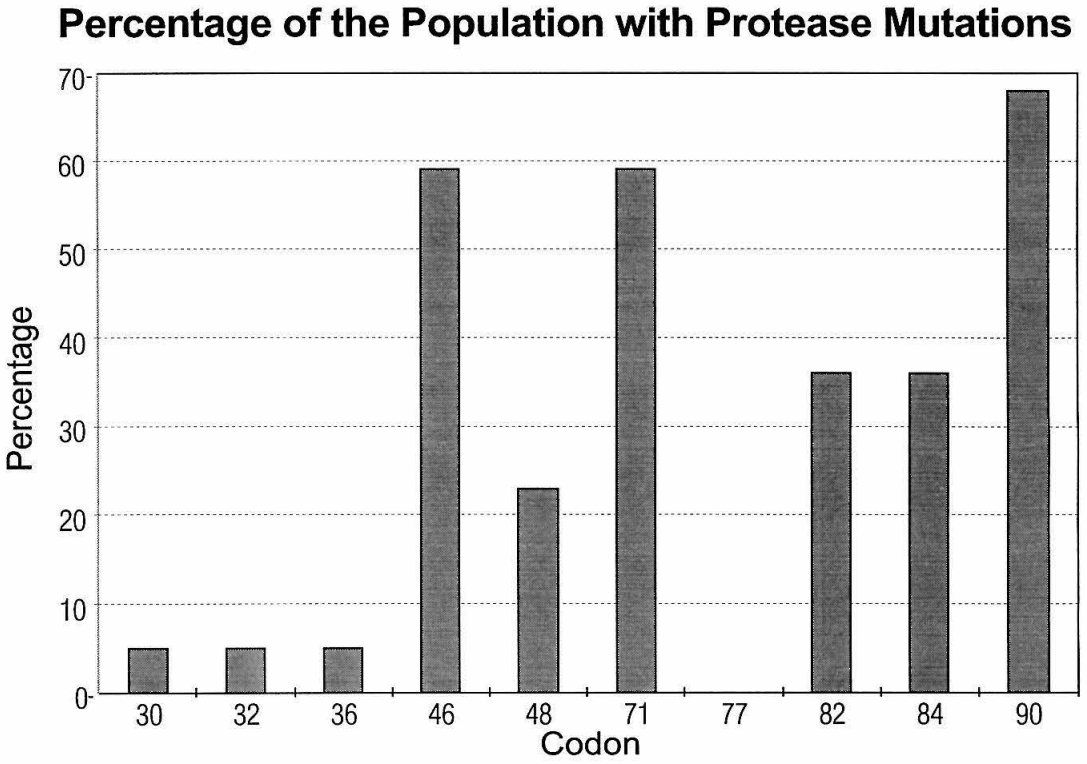

Figure 6.5: Percentage of the population with specified protease mutations.

6.7 Tables

Table 1. Characteristics of patients at baseline

Characteristic	N
Sex	
Male	60
Female	7
Mean age (yrs)	43
Median CD4+ Tcell cells/mm3	254
Median CD8+ Tcell cells/mm3	973
Prior opportunistic infection	14

Table 6.1: Baseline characteristics of patients

Antivirals taken previous to salvage therapy

Patient	AZT	DDI	DDC	D4T	3TC	Indinavir	Saquinavir	Ritonavir
1	x	x		x	x	x		
2	x	x		x	x	x		
3	x	x		x	x	x	x	x
4	x	x		x	x	x		
5	x				x	x	x	
6	x			x	x		x	x
7					x	x	x	
8	x	x		x	x	x	x	x
9	x			x	x	x	x	x
10	x				x	x	x	x
11	x				x	x	x	
12	x				x	x	x	
13				x	x		x	x
14	x				x	x	x	
15	x	x		x	x	x	x	x
16	x	x			x	x	x	
17	x				x	x		
18	x	x		x	x	x		
19	x			x	x	x		x
20	x	x	x	x	x	x	x	x
21	x				x	x		
22	x			x	x	x		
23	x	x		x	x	x	x	
24	x			x	x	x		
25	x			x	x	x	x	
26	x			x	x	x	x	
27	x	x		x		x		
28				x	x	x	x	x
29	x			x	x	x		
30	x			x	x	x		
31	x			x	x	x		
32	x	x		x	x	x	x	x
33	x		x		x		x	x
34	x			x	x		x	x
35	x			x	x	x		
36	x		x		x	x		

Table 6.2: Antivirals taken previous to salvage therapy

Antivirals Taken Previous to Salvage Therapy (cont.)

Patient	AZT	DDI	DDC	D4T	3TC	Indinavir	Saquinavir	Ritonavir
37	x		x	x	x		x	
38	x				x	x		
39	x	x		x	x	x		
40	x	x			x	x	x	
41	x	x		x		x	x	x
42	x	x	x	x	x	x	x	
43	x			x	x	x	x	x
44	x	x					x	
45	x				x	x		
46	x			x	x	x	x	
47	x			x	x	x	x	x
48	x			x	x	x	x	
49	x			x	x	x		
50	x			x	x	x		
51	x			x	x	x		
52	x			x	x			x
53	x		x		x			x
54	x				x	x	x	
55	x	x		x	x	x		
56	x	x		x	x	x		x
57	x	x	x	x	x	x	x	
58	x				x	x	x	x
59	x			x		x	x	x
60	x			x	x		x	x
61	x			x	x	x	x	
62	x	x		x	x		x	
63	x			x	x	x		x
64	x				x		x	
65	x	x	x	x	x	x		
66	x			x	x		x	
67	x	x		x	x	x	x	
Totals	64	23	8	48	63	55	38	23

Summary statistics for time until failure in days

Quantile	Point Estimate	95% Confidence Interval	
75%	206	118	427
50%	87	77	118
25%	66	54	77
Mean: 153.765		Standard Error 17.819	

Table 6.3: Days until failure by quantile

Log Viral Load Over Time

Time	Mean	Std. Err.	Difference	p-value
Baseline (Time 0)	3.52	0.189	n/a	n/a
Salvage time 1	2.97	0.185	-0.55	0.05
Salvage time 2	2.95	0.19	-0.57	0.1
Salvage time 3	3.16	0.36	-0.36	0.14

Table 6.4: Means and standard errors of log viral load over time

Square Root CD4+ Tcell Counts Over Time

Time	Mean	Std. Err.	Difference	p-value
Baseline (Time 0)	14.71	0.8385	n/a	n/a
Salvage time 1	15.6	0.8361	0.8969	0.4498
Salvage time 2	16.1	0.8765	1.3608	0.2634
Salvage time 3	16.04	0.8854	1.3295	0.2771

Table 6.5: Means and standard errors of CD4 T cell counts over time

Baseline Characteristics of Patients 1, 11, 35, 36,55, and 67

Characteristic	Mean	Median
CD4 T-cells/mm3	507.14	460
Viral load copies/ml	131.57	94
Number of previous drugs	4.5	4

Table 6.6: Baseline characteristics of the six patients who did not fail salvage therapy

Chapter 7 Outpatient Treatment Costs of HIV/AIDS in the Era of HAART

Abstract

For the first time since the start of the epidemic, the incidence of morbidity and mortality due to HIV/AIDS has decreased [4]. This decrease is attributed to the widespread use of antiretrovirals taken in combination, often called HAART (highly active anti retroviral therapy) [10]. HAART, for the purposes of this study, is defined as a combination of three or more antiretroviral agents from at least two classes[1]. Based on the projections of decreasing AIDS cases and subsequent reductions in hospitalizations, the costs attributed to HIV/AIDS are expected to decrease. The majority of the cost savings comes from the reductions in hospital visits. However, little is known about the long-term costs and effectiveness of HAART. This study looks at the costs of HIV/AIDS in the HAART era from 1995 to 1998 and compares it to the cost of therapy before HAART, 1988-1994 and to previous cost estimates in the pre-HAART era. The analysis shows that although HAART is more expensive to implement than the therapy available before HAART, there is a corresponding decrease in hospitalizations of HIV infected patients. Despite the decreased hospitalizations, the cost of treating patients with HAART is more expensive than the standard treatment patients were given previously. The incremental cost of HAART per year of life saved is estimated at $101,000 assuming HAART extends survival by four years. The incremental cost of HAART compares similarly with other treatments such as coronary artery bypass surgery and prostate-specific antigen screening.

[1]There are four classes of antiretrovirals, nucleoside, non-nucleoside, nucleotide, and protease inhibitors.

7.1 Introduction

The use of combinations of antiretrovirals, often called Highly Active Anti Retroviral Therapy (HAART), to treat HIV-1 infected patients has dramatically reduced morbidity and given longer life in HIV infected patients [4, 10]. HAART is defined here as the use of three or more antiretroviral agents from two different classes used in combination to treat HIV-1 infection. Although HAART has offered hope, is it cost-effective? HAART is more expensive to implement than previous treatment. Antiretrovirals are very expensive; a monthly dose of a single protease inhibitor can cost up to $600 per month. Due to the frequency and severity of side effects, patients need to be monitored frequently for blood imbalances. Patients also need expensive lab tests such as viral load and viral genotypic testing to determine if the virus has mutated to a drug resistant strain. Viral mutations can frequently lead to drug resistance and hamper efforts to control viral replication and subsequent disease progression. Frequent monitoring and laboratory testing is also expensive, viral load testing costs $200, viral genotyping costs $500 and viral phenotyping costs $1,000. Further, patients are also living longer and starting treatment earlier in the course of infection, leading to increased lifetime costs of treating HIV. However, patients on HAART have decreased incidence of AIDS defining opportunistic infections and decreased number of hospitalizations. Since hospitalizations and the AIDS stage comprise 58 percent of the lifetime costs of treating AIDS [3], prevention of these occurrences can have a significant effect on the cost of treatment. Recent reports have shown a dramatic decrease in the number of hospitalizations of patients on HAART. However, if the virus is not eradicated, then patients are still at risk of progression and this result may represent a short-term cost deferrment.

A recent paper [76] has shown that patients on long-term HAART are beginning to fail, that is, patients are beginning to progress to an AIDS defining event such as an opportunistic infection. As more patients fail HAART, the incidence of AIDS will increase as will the mortality rate due to HIV. As the rate of opportunistic infections increase, so will the number of hospitalizations. The increase in the hospitalization

rate will necessarily increase the cost of treating HIV infected patients and reduce the cost-savings gained by HAART. Since patients are living longer, starting treatment earlier in the course of their infection, and require more frequent monitoring and laboratory work, one would expect that the cost of treating patients with HAART will be more expensive than the treatment given to patients before HAART.

This paper looks at two groups of patients. The first group of patients were seen in an outpatient clinic from 1988-1994, the pre-HAART years. The second group of patients were seen at the same outpatient clinic during 1995-1998 and treated with HAART. With these two groups one can look at the cost patterns over time between treatment regimes. It was found that the number of doctor visits has been steadily increasing from 1988-1998 with the greatest increase from 1994 to 1995. The trend is similar with the number of laboratory tests such as CD4 T cell counts, complete blood counts and blood chemical screening. In the HAART regime, 1995-1998, the number of CD4 T cell count tests and viral load testing increased dramatically as would be expected because of the increased need for monitoring patients. The total number of hospitalizations increased from 1988 until 1994. In 1995 there was a dramatic decrease in hospitalizations. This trend was sustained in 1998. To test whether the decrease in hospitalizations was enough to offset the cost of increased monitoring, doctor visits and medication, the incremental cost per year of life saved was estimated for varying longevities. The total lifetime cost of treating HIV/AIDS was also estimated. It was found that as HAART increased survival times, the incremental cost per month gained decreased. If HAART had no effect on survival or on hospitalization, the incremental cost for HAART was $289,000, 95 percent confidence interval [$251,000-$330,000]. However, if HAART increased survival by only six months, the cost per month gained was $51,000 [$44,000-$58,000]. If HAART increased survival by four years, the cost per month gained was $8,400 [$7,300-$9,500]. Assuming HAART extended life for four years, the incremental cost of HAART per year of life saved was $101,000 [$88,000-$114,000].

The lifetime direct costs of treating an HIV infected patient before HAART was estimated at $132,000 [$123,000-$141,000]. The lifetime direct costs of treating HIV

with HAART ranged from $289,000 to $402,000 depending on the increased survival and the assumption that HAART has no sustained decrease in hospitalization. If HAART has a permanent decrease in hospitalizations of 30 percent, then the cost per year of life saved is only $96,000 [$82,000-$109,000].

7.2 Methods

There are two distinct regimes in the treatment of HIV: HAART and pre-HAART. The pre-HAART regime extends from 1988-1994. This regime is defined from 1988 until 1994 when there existed antiretroviral medication in the form of nucleoside reverse transcriptase inhibitors, prophalaxis medication for opportunistic infections but before protease inhibitors and combination therapy. Before HAART it was estimated that the average time from seroconversion (detection of HIV antibodies) to AIDS was 10 years [6]. Patients progressed in a typical fashion with CD4 T cell counts decreasing in a monotonic pattern. Treatment in this era had only a transient effect on viral load and drug resistance was quickly established. The pre-HAART regime is substantially different from the HAART regimen whose therapy had a significant effect on CD4 T cell counts and viral load. Data on 210 patients who were seen in an outpatient clinic from 1988-1994 were collected by a retrospective chart review. Patients were chosen by a random sample of all patients in the HIV clinic. The only selection criteria was seropositivity. Data were collected on number of doctor visits, number of laboratory tests, number of hospitalizations, insurance status, costs and disease stage.

Hellinger estimated the lifetime costs of treating an HIV infected person in 1992 using the AIDS Cost and Services Utilization Study (ACSUS). This is a very detailed study on the various aspects of direct costs. The data collected from the outpatient clinic were compared to the results of Hellinger's study.

With the introduction of HAART in 1995, patients saw dramatic reductions in viral load (number of copies of HIV RNA in the peripheral blood) and substantial increases in CD4 T cell counts [33, 34, 35]. For the first time in the epidemic there

was hope. It has been hypothesized that combination treatment could suppress viral replication long enough to allow the immune system to recover or reconstitute [11]. By constructing a nonparametric density estimate of CD4 T cell counts from patients on HAART and comparing it to the CD4 distribution of uninfected patients, this hypothesis was tested. The resulting distribution is significantly below that of the uninfected population, suggesting that even with HAART, patients CD4 T cell counts do not increase to normal levels with HAART and thus patients are still immunocompromised [77]. It has also been hypothesized that utilizing antiretrovirals in combination would create a genetic barrier high enough so that HIV could not mutate around the drugs and cause resistance [11]. Data from Ramirez 1998 [78] differs substantially from this hypothesis. Viral isolates that were genotyped found high levels of resistance in patients on HAART. In a subset of patients who had failed primary combination therapy, it was found that subsequent salvage therapy failed to suppress viral replication. This result reinforces the need for caution and strategic treatment in selecting antiretroviral agents. Although resistance is a major problem, patients on HAART have fewer hospitalizations and compared to the population before HAART was available. It was also found that patients who were naive, not experienced with antiretroviral therapy, had a significantly greater time until AIDS than patients who were pre-treated. For patients who have been diagnosed with an opportunistic infection, naive status did not have a significant effect on time until the next opportunistic infection. This result suggests that patients should seek early and aggressive treatments. Since HAART is relatively new and HIV has a long latency period, it is too early to know the full effects of HAART on long-term survival for asymptomatic people. To fully address all of the cost issues, it would be necessary to have the full survival distributions of all patients on HAART. In that way, one could know how much HAART increased the time until AIDS. Since the exact survival is not known, the direct cost of treating patients with HAART is estimated for different levels of longevity.

Data were collected on 258 patients in the same outpatient clinic who were on HAART from 1995-1998. Patients were selected by a random sample. For each

person, data were gathered on number of doctor visits, number of laboratory tests, number of CD4 T cell count tests, number of viral load tests, number of hospitalizations, insurance status, cost, and disease stage.

Data for both groups were aggregated by year to assess the trends on a yearly basis. Average yearly costs for doctor visits and laboratory tests were estimated as well as average yearly costs for medication giving the total outpatient cost per year. Total number of hospitalizations per year are also given. All dollars are in 1995 dollars.

7.3 Results

7.3.1 Direct Costs of the Pre-HAART Era

In 1992, Hellinger used self-reported charge data from the ACSUS (AIDS Cost and Service Utilization Study) to estimate the lifetime costs of HIV. He calculated the average monthly charges for patients in four stages of HIV infection. The stages were classified by CD4 T cell count and the presence of an AIDS defining opportunistic infection. Hellinger estimated that in the pre-HAART era, outpatient costs including drug costs were $4,771 per year. Hellinger estimated that patients spend 10.31 years before progressing to AIDS spending a $33,061 in outpatient costs and $50,174 in direct total costs. Direct total costs include inpatient costs and long-term care. Once diagnosed with AIDS, Hellinger estimated it was 25 months before the patient died. The outpatient cost for the AIDS stage per person was $16,125 and the direct total cost was $69,100. Combining the two total costs yields $119,274 as the lifetime cost of treating each HIV infected patient from seroconversion until death in the pre-HAART era.

Data from the outpatient cohort from 1988-1994 are consistent with the findings of Hellinger. Data was gathered on outpatient costs only. Data on hospitalization, home health care costs, and long term care were unavailable. In 1988 patient visited the doctor an average of 3.63 times. The rate was relatively stable throughout the

era but slightly increasing. By 1994, patients had an average of 4.90 doctor visits. In 1988 patients had an average of 9 laboratory tests performed, two of which were CD4 T cell count tests. Viral load testing was not widely available until 1995. By 1994, patients had an average of 12.1 laboratory tests, three of which were CD4 T cell count tests. Doctor visits and laboratory test comprise the majority of outpatient costs. Table 7.1 lists the number of doctor visits and the number of laboratory tests performed each year.

Outpatient costs were estimated based on charge data. Costs were estimated for each year in the pre-HAART era. It was estimated that each patient spent $1,098 on average in 1988 on outpatient expenditures,. In 1994 patients spent, on average, $1,506.25 on outpatient care. Note that the numbers does not include the cost of medication.

In the pre-HAART era nucleoside reverse transcriptase inhibitors were available to inhibit the replication of HIV. There also existed prophalactic medication for certain opportunistic infections. Medication costs were estimated by taking the average cost of the most frequently prescribed drug regimen. In the pre-HAART era the medication regimen included AZT (anti-HIV medicine), Bactrim (Pneumocystis Carinii pneumonia prophalactic, Diflucan (thrush and fungal infections prophalaxis), Marinol (combat side effects, stimulate appetite), and Acyclovir (anti-herpes drug). The average cost of this regimen was $295 [$249-$341] per month or $3,500 [$3,000-$4,100] per year. Adding drug costs with outpatient costs yields total outpatient direct costs per year and are given in Table 7.2. The average outpatient cost in the pre-HAART era was $1,400 [$1,200-$1,600]. When drugs were added to this cost, the average total outpatient cost was $4,900 [$4,200-$5,600].

Unfortunately, data on inpatient, home health care and long-term care were unavailable for this outpatient group. Data were available on the total number of hospitalizations. In 1988 there was 143 hospitalizations, yielding 1,269 hospital days. In 1994 there were 275 hospitalizations, yielding 1,763 hospital days. The total number of hospitalization are given in Table 7.4. Since the hospital charts are unavailable, the cost for each of these hospitalizations are unknown.

Survival analysis with AIDS as the primary endpoint was performed on the clinical data with similar results to Hellinger. The median time from seroconversion until AIDS is 10.2 years. Median time from AIDS until death was 2.3 years. Hellinger estimates that patient takes 10.31 years from seroconversion until AIDS as defined by the presence of an opportunistic infection. Once a patient has an opportunistic infection, it takes 2.08 years until death. Outpatient cost estimates and the survival estimates from the outpatient data are consistent with that of Hellinger's estimates from ACSUS. Using Hellinger's estimates for inpatient, home health care, and long-term care costs; lifetime average cost to treat an HIV-infected patient in the pre-HAART era was $132,000 [$123,000-$141,000].

7.3.2 Direct Costs of HAART

As HAART is relatively new, little is known about the long term cost and effectiveness of HAART. Charge data were gathered on outpatient expenditures. Data on hospitalization, home health care, and long-term care cost were unavailable. Patients on HAART had an average of 6.5 doctor visits in 1995. This number increased to 9.67 visits in 1996 and averaged 9.7 visits in 1998. The number of doctor visits per year are given in Table 7.1. The increase in doctor visits directly relates to the increased complexity of the HAART regimens compared with the therapeutic regimens in the pre-HAART era. The severity and frequency of side effects necessitate increased monitoring of patients. In 1995 the average number of laboratory tests performed was 16.3 tests. This number rose to 27.17 tests in 1996 and increased to 27.30 tests in 1998. The increase in testing is not only a function of side effects but also of resistance monitoring. Drug resistant HIV is a critical issue. Viral mutations that confer drug resistance can hamper efforts to control viremia and increase the likelihood of disease progression. Frequent monitoring and laboratory tests such as viral load testing and viral genotypic testing can detect emerging resistance. At the first sign of resistance the patient's antiretroviral regimen was modified to prevent high level resistance. In 1995 patients had an average of 3.07 CD4 T cell count tests performed. In 1996 the

number rose to 4.89 tests and by 1998 patients had an average of 5.56 CD4 T cell count tests performed. Viral load testing became available in 1995 and became widely used in HIV treatment in 1996. In 1995 patients on HAART had an average of 1.27 viral load tests performed. In 1996, patients had 5.53 tests performed and by 1998, patients had 5.97 viral load tests performed; see Table 7.3. Genotypic testing is very new and very expensive. Patients went from an average of 0.5 tests in 1997 to 1.41 tests in 1998.

Viral load testing costs $200, CD4 T cell count testing costs $165, and viral genotypic testing costs $500. The expense and frequency of these monitoring tests add substantially to the direct outpatient costs of treating patients with HAART. In 1995 when HAART was first available, the average outpatient cost to treat a single patient was $2,111.25. This figure rose substantially in 1996, when viral load testing and HAART drugs were more widely available, to $3,875.35. By 1998, the average outpatient cost was $4,033.55. These figures do not include the price of medication.

Antiretroviral medications are very expensive. These drugs are expensive to manufacture and many are unstable and thus have a very short shelf life. Because of this and the fact that the research and development costs were enormous, it is not likely that the price for the drugs will come down substantially in the near future. Average medications costs were estimated by taking the average cost of the most prescribed drug regimen. In the HAART era the most prescribed drug regimen was AZT, 3TC, Indinavir (all three anti-HIV drugs), Bactrim (PCP prophalaxis), Zithromax (to prevent Mycobacterium avium complex), Acyclovir (to treat herpes zoster), Diflucan (to prevent fungal infections), Marinol (appetite stimulant), Zoloft (antidepressant), Oxandrin (to prevent wasting syndrome), and testoterone (to prevent wasting syndrome). The average monthly cost of this regimen was $2,100 [$1,800-$2,300]. Assuming no change in the regimen, the average yearly cost for medication was $24,600 [$21,500-$28,000].

Adding the cost of medication to the outpatient costs yield the total average outpatient costs for HAART. The average total outpatient cost for HAART was $28,100 [$24,000-$32,000]. These costs did not include hospitalizations, psychological

counseling, transportation costs, non-traditional or holistic medicine, or home health care costs.

Use of these medications long-term will significantly increase cost. Assuming that the drugs are effective and non-toxic for everyone, patients will incur more outpatient expenses in the form of outpatient visits and medication costs. These costs are substantially greater than that of the previous treatment regime. However, the greatest costs occur in the AIDS stage when hospitalizations are more likely. If HAART can prevent patients from needing to be hospitalized, then HAART could have a cost savings over the previous regime. When one looks at the total number of hospitalizations per year in the HAART regime, one can see a substantial decrease. In 1995 there were 53 total hospitalizations. The number of hospitalizations remained relatively stable, and by 1998 there were 74 total hospitalizations. The average number of hospitalization in the HAART regime was 71, yielding an average of 439 hospitl days. See Table 7.4. Compared with 1,607 hospital days in the pre-HAART era suggests that the number of hospitalizations as well as the length of the hospital stay decreased under HAART. With the introduction of prophalactic medications for MAC (Mycobacterium aviium complex) and CMV (Cytomegalovirus), physicians were able to prevent some of the most costly opportunistic infections. Earlier studies have shown that PCP (Pneumocystis carinii pneumonia) prohpalaxis has decreased the incidence of PCP which was the most common opportunistic infection. As hospitalizations generally occur after the onset of an opportunistic infection, and there has not been a substantial change in the treatment of opportunistic infections in the past 7 years, there should not be a significant difference in inpatient treatment between regimes. However, as previous results have shown, HAART cannot fully prevent disease progression and opportunistic infections. Thus, the reductions in hospitalizations could be transient and, in fact, act as a cost delay. However, if HAART can prevent certain opportunistic infection that require lengthy hospital stays, there could be a cost-savings invloved. The analysis will take into account two scenarious, one where HAART only has a transient effect on inpatient and long-term costs and the second where HAART has a 30 percent reduction in inpatient costs due to decreased hospital

length. Thus under one regime, patients will spend an average of $70,000 on inpatient costs and the other $49,000, holding all else constant.

Lifetime costs of treating patients on HAART depends on many factors; the efficacy of the drug at preventing disease progression, toxicity, long-term side effects, resistance, and patient compliance. Unfortunately, HAART is relatively new and the true long-term effects are unknown. Previous survival analysis [76] has shown that HAART cannot fully prevent disease progression. Other studies [14, 15] have shown that HIV is not eradicated therefore, patients are subject to viral rebound and disease progression. Given that patients will eventually fail but the exact failure time is not known, the cost per month of life gained can be calculated for various survival times.

If HAART has no effect on prolonging life but decreases hospitalization costs by 30 percent, then the total lifetime direct costs of treating an HIV infected patient is $400,000. If HAART does not decrease hospitalization costs and has no effect on survival, the lifetime cost is $421,000. If HAART is effective in prolonging life for four years, then the lifetime cost of treating that patient with HAART is $534,000. If HAART has a positive effect on survival but no effect on cost-savings and increases survival by 6 months, then the cost per month gained is $51,000. If HAART prolongs life by 12 months, then the cost per month gained is $27,000. If HAART increases survival by 48 months, the cost per month gained is only $8,400. The more HAART increases survival, the less the cost per month of life gained. Table 7.5 lists the lifetime direct costs of treating HIV/AIDS and the cost per month gained for various survival times.

Because incremental costs are widely used to compare different medical interventions, the incremental cost per year of life saved was estimated for HAART. If HAART extends life by four years, then the incremental cost per year of life is $101,000. The incremental cost of prostate-specific antigen screening is $113,000 per year saved and the incremental costs of coronary artery bypass surgery is also $113,000 per year saved. Thus HAART compares favorably to other common medical interventions. However, the number of HIV infected individuals is greater than candidates for other interventions. According to the latest Centers for Disease Control (CDC) estimates,

650,000 to 900,000 Americans are living with HIV and at least 40,000 new infections occur each year [4]. If every HIV-infected individual was put on HAART, the effects on the health care system could be staggering.

HIV affects a young population relative the the population who are candidates for coronary artery bypass surgery. These people are in their most productive work years. If HAART can keep these people working and productive memebers of society, the cost of HAART, in terms of society is actually much lower than $534,000.

7.4 Discussion

This paper has looked at treatment trends in HIV from the years 1988-1998. There are two distinct regimes in this period; pre-HAART and HAART. Over time, costs have been increasing. The increasing costs are due to the increased monitoring necessary to implement HAART as well as the costs of HAART itself. Although HAART is more expensive than the pre-HAART treatment, significant cost saving were seen due to the reductions in hospitalizations. Reductions in hospitalizations are due, for the most part, by the reduction in the incidence of AIDS defining opportunistic infections.

Failure of HAART to eradicate the virus leaves patient vulnerable to resistance and eventual resurgence of HIV [14, 15]. It is important to know that HIV is not over. As patients begin to progress, the incidence of AIDS defining opportunistic infections and hospitalizations and cost will increase.

To assess the impact of the cost of HAART on the health care system, it is necessary to look at the cost to treat all HIV infected patients. Before effective treatment many patients did not come in for testing or treatment. Thus, they did not affect the cost of treating HIV/AIDS until they became very sick. With HAART there is an effective treatment that can prolong life if taken early enough. Now there is an incentive for patients who were previously untreated to start taking HAART. This could increase the CDC figures of the number of HIV infected people in the United States. The more people seek treatment, the higher the total cost on the health care system.

As more patients go in Health Maintenance Organizations (HMO's), it will be interesting to see how they respond. HIV treatment will be along defined clinical guidelines and benefits given based on predicted costs. Managing costs in a disease that has so far been unmanageable remains a challenge to those in the HMO industry. HIV treatment is very complex and dynamic. The very nature of treatment has changed as new treatment and technologies become available. As has been shown earlier, even a lifetime capitation rate of $250,000 would be insufficient to care for an HIV infected individual on HAART.

Unfortunately, there is insufficient data available to calculate exactly how long HAART delays disease progression. As HIV is at least a ten year disease, and HAART has only been available for four years, it is not possible to predict when the majority of patients on HAART will progress. Detailed data needs to be collected prospectively on patients on HAART. Data especially on newly infected individuals who start early and aggressive treatment is especially needed as these patients have the best chance of succeeding with HAART. Data needs to be collected on their antiretroviral treatment regimens, prior treatment regimens, side effects, viral load, CD4 T cell count, CD8 T cell count evolution of viral genotype and phenotype, genotype of the individual, age, weight, and symptomology. In several years, one could know the true cost of HAART and the long-term efficacy of this regimen. There is need for better drugs that are easier to take and less likely to confer cross-resistance. HAART as it stands is not enough. We are still far from a cure and policy makers need to be prepared for the eventuality of dramatically increased HIV/AIDS care costs.

7.5 Tables

Average Number of Lab Tests and Doctor Visits

Doctor Visits		Lab Test (all)	
1988	3.63	**1988**	9.00
1989	4.27	**1989**	10.10
1990	4.81	**1990**	13.00
1991	4.80	**1991**	13.00
1992	4.60	**1992**	12.40
1993	4.85	**1993**	12.00
1994	4.90	**1994**	12.10
1995	6.50	**1995**	16.30
1996	9.67	**1996**	27.17
1997	10.00	**1997**	26.52
1998	9.70	**1998**	27.30

Table 7.1: Average number of doctor visits and laboratory tests per year

Average Outpatient Costs per Year

Year	Outpatient costs	Drug Costs	Total
1988	$1,098.00	3543.24	$4,641.24
1989	$1,248.25	3543.24	$4,791.49
1990	$1,546.17	3543.24	$5,089.41
1991	$1,545.00	3543.24	$5,088.24
1992	$1,493.63	3543.24	$5,036.87
1993	$1,506.25	3543.24	$5,049.49
1994	$1,517.00	3543.24	$5,060.24
1995	$2,111.25	24620.04	$26,731.29
1996	$3,875.35	24620.04	$28,495.39
1997	$3,921.70	24620.04	$28,541.74
1998	$4,033.55	24620.04	$28,653.59

Table 7.2: Average outpatient costs, drug costs, and total cost per year

Number of Monitoring Tests in the HAART Era

CD4 Count Test		Viral Load tests	
1995	3.07	**1995**	1.27
1996	4.89	**1996**	5.53
1997	5.06	**1997**	5.62
1998	5.56	**1998**	5.97

Table 7.3: Number of monitoring laboratory tests per year during HAART

Total Number of Hospital Visits per Year	
1988	143
1989	157
1990	172
1991	184
1992	279
1993	224
1994	275
1995	53
1996	82
1997	75
1998	74

Table 7.4: Total number of hospitalizations per year

Lifetime Direct Costs of Treating HIV/AIDS

	Total cost	95% CI	cost/ month gained	95% CI
Old Regime	$132,000			
HAART Regime-Decreased Hospitalizations				
0 month gain	$400,000	[353,000-449,000]	$268,000	[230,000-308,000]
6 month gain	$414,000	[365,000-466,000]	$47,000	[40,000-54,200]
12 month gain	$429,000	[378,000-481,000]	$24,750	[21,000-28,000]
24 month gain	$457,000	[402,000-513,000]	$13,542	[12,000-16,000]
36 month gain	$485,000	[402,000-513,000]	$9,806	[8,400-11,000]
48 month gain	$513,000	[451,000-577,000]	$7,938	[6,800-9,100]
HAART Regime-No Decrease in Hospitalizations				
0 month gain	$421,000	[374,000-470,000]	$289,000	[251,000-330,000]
6 month gain	$435,000	[386,000-487,000]	$50,500	[44,000-58,000]
12 month gain	$450,000	[399,000-502,000]	$26,500	[23,000-30,000]
24 month gain	$478,000	[423,000-534,000]	$14,417	[13,000-16,000]
36 month gain	$506,000	[447,000-566,000]	$10,389	[9,000-12,000]
48 month gain	$534,000	[472,000-598,000]	$8,375	[7,300-9,500]

Table 7.5: Lifetime direct costs and cost per month gained of using HAART to treat HIV/AIDS

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