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Patterns and associations with immunologic response in patients accessing ART in Khayelitsha

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ALFEOUS RUNDARE
(RNDALF001)

Supervisor: Dr Andrew Boulle

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DECLARATION

I, Rundare Alfeous (RNDALF001), hereby declare that the work on this dissertation is based on my original research and has not, in whole or in part, been submitted towards another degree, at this University or elsewhere. The University is empowered to reproduce either the whole or any portion of the contents for the purposes of research.

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ABSTRACT

Introduction

This study formed part of an existing prospective cohort study describing the outcomes of treatment of patients accessing ART in Khayelitsha. Despite the reported favorable outcomes in terms of immunologic responses, the actual variations in patterns of and associations with immunologic response over time among adult patients accessing the community based antiretroviral treatment programme in Khayelitsha are largely unknown.

The aim of the study

The aim of this study focused on describing the patterns of and associations with immunologic response, together with some of their subsequent outcomes among adult patients accessing community based antiretroviral treatment programme in Khayelitsha.

Study design and population

The analysis of this study formed part of an existing prospective cohort study describing the outcomes of antiretroviral treatment of patients in Khayelitsha. The study population included patients accessing ART in Khayelitsha, Cape Town, South Africa. A sample size of 400 HIV positive ART naïve patients was sufficiently powered for the analysis. The socio-demographic and clinical information required for the analysis was already captured, validated and entered in a database. Summary measures, logistic regressions, survival analysis, simple linear regression and population average models were used to make the analysis and report the findings.

Findings

There were 3373 patients included in the analysis, of which females constituted 70% (2358) of the study population. The median age was 32 years (IQR 28-38). Males were significantly older

than females and their median ages were 36 years (IQR 40-41) and 31 years (IQR 27-36) respectively. The greatest proportion of patients was between 25-34 years (51.3%).

The median baseline CD4 count increased from 92 cells/μl (IQR 38-149) to 282 cells/μl (IQR 200-313) after one year of ART. The CD4 cell count increase was the highest in the first 6 months-135 cells/μl (IQR 79-201) - and a more than 50% decline in this rate was observed in subsequent months. The greater increase was further achieved when viral load was suppressed at two time points and throughout the four years of follow up.

The effects of baseline CD4 cell count on CD4 cell increase was the same at each time point across all baseline categories but also higher in the first year than the subsequent years. At the first six month time point, fewer gains in CD4 cell count were associated with older age, male gender and higher baseline CD4 cell count ($P<0.05$) and these were -35.5 (95% CI -56.7; -14.4), -20.6 (95%CI -31; -10.2), and -14.5 (95% 8.1; 20.9) respectively. The gains in CD4 cell count at six months were associated with log 10 baseline viral load and the viral load at six months. These were 14.5 (95% CI 8.1; 20.9 and 25.8 (95%CI 11.2 40.4).

The analysis of CD4 cell count changes beyond six months was done using three different models. The first one was inclusive of all patients, the second model included those suppressed at successive time points and the last model focused on patients with viral load suppression throughout all the follow up time points. In all these scenarios, fewer gains in CD4 cell count were commonly associated with lower baseline CD4 cell count, older age, high previous CD4 cell count and male gender ($P<0.05$). The CD4 cell count gains were associated with log 10 baseline viral load, the WHO clinical stage 4 and current viral load. In fact even more gains were associated with suppressed viral load.

The frequency of discordant response was 4.2% in the first twelve months. The factors independently associated with the greatest risk of discordant response (that is patients who attained less than 50 cells/μl increase of CD4 cell count from baseline despite suppressing viral load to levels below 400 copies/ml) were of an older age and higher baseline CD4 cell count. The patients in the age range greater than 44 years old had a four fold (RR=4.3, 95% CI 4.3; 15.4) increased risk of having a discordant response compared to patients between the age of 14-24. The patients with baseline CD4 cell count greater than 150 cells/μl had the greatest risk of discordant response (RR=6.8, 95%CI 3.0; 15.7) compared to patients with baseline CD4 cell count below 50 cells/μl.

The clinical progression to death between discordant and non discordant response beyond 12 months was the same (P=0.11). The CD4 cell count beyond 12 months was the only variable associated with subsequent survival (P<0.01).

Discussion

Gender, age and baseline markers of disease progression (CD4 count, WHO stage and viral load) are strong determinants of CD4 cell response on ART in patients in the Khayelitsha HIV programme.

Males in this cohort had a lower median baseline CD4 cell count and subsequent gains in CD4 cell count compared to females. This has been attributed to differences in immunological response between males and females (Finkel et al. 2003). In the cohort there was a biphasic pattern of CD4 recovery on ART: there was an initial rapid recovery of CD4 cells in the first 6 months, followed by a more gradual recovery. Importantly there was continued gain in CD4 count throughout the 48 month period, suggesting that patients may potentially improve to CD4

count values in the normal range if they continue ART with suppressed viral loads for sufficient time (Mocroft et al. 2007).

CD4 gains were more pronounced in patients with viral load suppression throughout the follow up time, followed by gains in patients with suppressed viral load at two successive time points. Although rates of increase in CD4 cell counts appeared to decline with subsequent years of ART, the effect of the baseline CD4 cell count on the rates of CD4 cell change over time persisted. This observation supports the possibility of attaining normal CD4 cell count similar to healthy individuals.

The frequency of discordant response was low in our data. It is in fact less common in low income countries as compared to high income countries (Lawn et al. 2006). Discordant response in the first 12 months did not have an impact beyond 12 months on both CD4 cell count change over time and subsequent outcome (death) in the Khayelitsha HIV programme. The patterns of immune response in the Khayelitsha HIV programme are similar to those seen in patients from high income countries. As long as viral suppression is maintained, immune recovery is possible in patients with the advanced disease

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ABBREVIATIONS

AIDS	Acquired immune deficiency syndrome
ART	Antiretroviral therapy
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
MSF	Medicine Sans Frontières (Khayelitsha study)
TB	Tuberculosis
WHO	World Health Organization
UNAIDS	Joint United Nations on HIV/AIDS
OP	Opportunistic infections
MAC	*Mycobacterium avium* complex infections.
HCV	Hepatitis C virus
HCV	Hepatitis G virus
HBC	Hepatitis B virus
cART	combined Antiretroviral therapy
IRIS	Immune reconstitution inflammatory syndrome
µl	Micro-litre
RNA	Ribonucleic Acid
DNA	Deoxy-ribonucleic acid
AZT	Zidovudine
3TC	Lamivudine
ART-LINC	Antiretroviral Therapy in Lower Income Countries Collaboration

CHAPTER 1 - INTRODUCTION

1.1 Background

This project arose out of a request from the Khayelitsha antiretroviral treatment (ART) programme, to assist in describing the CD4 count changes over time in the treated cohort of adult patients. This chapter provides the background to the project and the context in which it operates, as well as the human immunodeficiency syndrome (HIV) epidemic, and the role of CD4+ lymphocytes and the monitoring of CD4+ lymphocyte cell counts.

1.1.1 Epidemiology and burden of HIV/AIDS

1.1.1.1 *The global HIV/AIDS epidemic*

According to UNAIDS (2006), it is estimated that a total of 39.5 million people were living with HIV in 2006 (2.6 million more than in 2004) (Fig 1). Also, the number of new infections in 2006 rose to 4.3 million in 2006 (400 000 more than in 2004). In 2006, 2.6 million AIDS related deaths were recorded.

Clearly, Sub-Saharan Africa remains the most affected region in the world. Equally important, not all African countries portray the same trend in terms of HIV prevalence and subsequent AIDS related mortalities. It is therefore important to take cognisance of the large differences in Sub-Saharan countries (Asamoah-Odei et al. 2004). In 2006, two thirds of all people living with HIV lived in the Sub-Saharan region (that is 24.7 million people) (UNAIDS 2006).

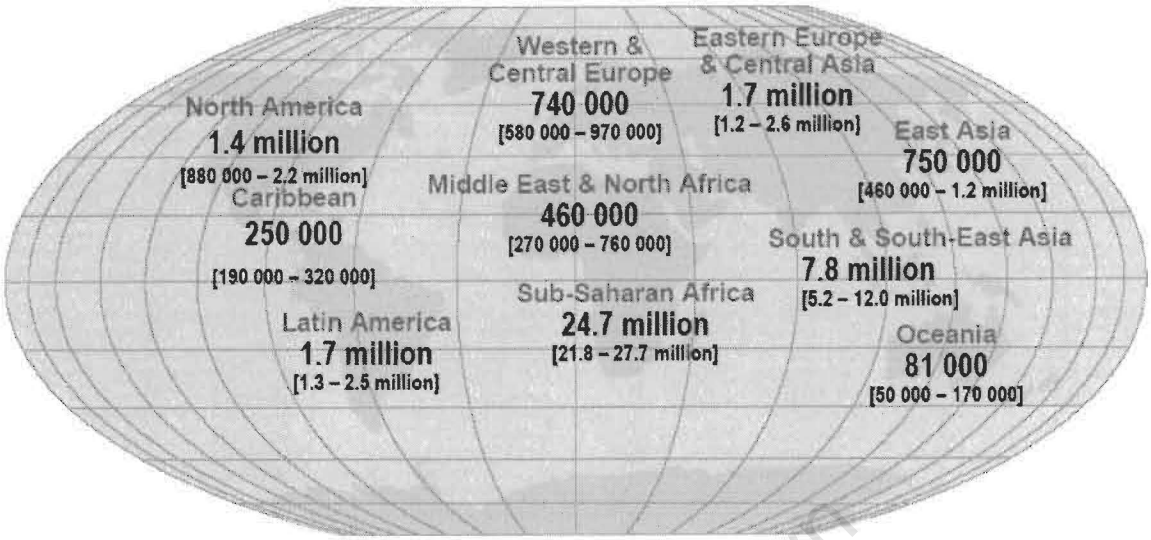


Figure 1 : Adults and children estimated to be living with HIV/AIDS, 2006*

According to the UNAIDS (2006) report, globally and in every region, more adult women (15 years or older) compared to males in the same age group are now living with HIV. At the same time, 17.7 million women are living with HIV, an apparent increase of over one million compared with 2004 estimates. The report also indicated an increase in access to treatment in recent years. It also highlighted a gain of two million life years since 2002 in low and middle income countries through the expanded provision of antiretroviral treatment.

1.1.1.2 *HIV/AIDS epidemic in South Africa*

In 2005, it was estimated that 5.5 million people of the South African population, including 240 000 children younger than 15 years, were living with HIV.

* Adapted from UNAIDS, 2006

In 2006, the Department of Health undertook a study based on a sample of 33,033 women who attended 1,415 antenatal clinics across all nine provinces. The findings of the study estimated that 29.1% of pregnant women were living with HIV in South Africa. The study also estimated that HIV prevalence among the antenatal clinic attendees by province was 39.1% for KwaZulu-Natal followed, by Mpumalanga (32.1%), Free State (31.1%), Gauteng (30.8%), North West (29%), Eastern Cape (29%), Limpopo (20.7%), Northern Cape (15.6%) and Western Cape (15.2%) (Department of Health 2006).

1.1.1.3 HIV/*AIDS epidemic in Khayelitsha*

Khayelitsha is in the Western Cape Province and is located 30 kilometers from Cape Town, South Africa, and is known to be one of the fastest growing townships in South Africa. It is estimated to have more than 500,000 inhabitants, of whom 50% are unemployed and more than 70% reside in informal settlements.

The 2006 Annual Activity Report, for the Khayelitsha HIV programme reported that Khayelitsha had the largest number of people living with HIV in the Western Cape. The report highlighted that between 1999 and 2005 the Khayelitsha antenatal HIV prevalence doubled from 15% to 30%. This prevalence was similar to the national average, but higher than the provincial average of 17.5%.

1.1.1.4 *Description of the Khayelitsha ARV Programme*

According to aforesaid report, the Khayelitsha HIV programme was a brain child of Western Cape Department of Health and the Medecins Sans Frontières (MSF). The aim of the programme was "to demonstrate effectiveness, feasibility and acceptability of treating persons infected with HIV, including the provision of antiretroviral therapy (ART), in a primary health care setting" (Annual Khayelitsha Activity report 2006:1). The report indicated that from this programme, Khayelitsha emerged as one of the sentinel sites for monitoring antiretroviral therapy (ART).

In Khayelitsha, three clinics at different sites were opened to provide appropriate counseling, support, prophylaxis treatment, screening and referral for conditions related to HIV/AIDS. The advent of the HIV epidemic has increased the burden of TB significantly. Khayelitsha has the highest TB case finding and accounts for 23% of the caseload for the Metropole region. In 2003, TB services integrated HIV initiatives such as the VCT. Patients testing positive were referred for CD4 cell count and to HIV care. Amongst patients entering HIV care, those referred from TB constituted patients with the lowest CD4 cell counts.

As will be discussed in the next section, the HIV directly infects and subsequently destroys the white blood cells which function to protect the body from any pathogen invasion. This weakens the immune system. Against this background, the provision of HAART had favorable outcomes in terms of the immune response specifically for the Khayelitsha cohort in the long run. The median baseline CD4 count in 2004 was 43 cells/μl (IQR 13-94) and the proportion of patients with baseline CD4 cell count less than

50 cells/μl was 55% (Coetzee et al. 2004). A secondary analysis by Van Cutsem et al. (2007) for the same data used by Coetzee et al. (2004) three years earlier, showed an increase in baseline CD4 cell count and proportion of patients with baseline CD4 cell count <50 cells/μl which were 87 cells/μl (IQR 35 146) and 33% respectively.

The Khayelitsha Activity report (2006) was also reported that the ART scaling up programme between 2002 and 2005 had a significant impact on the baseline CD4 cell count. The baseline CD4 cell count increased from 48 in 2001 to 105 cells/μl in 2005. The rapid increase in baseline CD4 cell count reflected an increase in enrollment of patients accessing treatment with less immune suppression (The Western Cape ARV Programme Monitoring Report, 2006).

Despite these reported favorable immunologic responses, the actual variations of patterns and associations with immunologic response over time among adult patients accessing community based antiretroviral treatment programme in Khayelitsha are not known.

1.1.2 HIV/AIDS and the immune system

The immune system is composed of a complex network of independent cells that collectively work together to defend the body against bacterial, fungal, parasitic and viral infections and from the growth of tumor cells (Engenderhealth 2005).

Goepfert (2002) describes the human defense against infections to include surface barriers, innate defenses, and adaptive (or acquired) responses. In so far as the HIV immunopathogenesis is concerned, the author suggests that the adaptive or acquired response takes the center stage. When HIV infects a cell, mechanisms of the body`s

immune defense system are stimulated. These comprise the B-cell (humoral) and T-cell (cell mediated) immune mechanisms (Goepfert 2002).

The T cells mainly include the CD4+ cells, viewed as being responsible for the adaptive immune responses (Goepfert 2002). The author also discusses how CD4+ cells recognize foreign antigens bound to host proteins and aid B cells in the production of antibodies. According to the author antibodies neutralize cell-free viruses, thereby preventing subsequent viral infection of target cells in the body. The CD4+ cells also participate in the lyses of infected cells by recruiting natural killer (NK) cells in a process termed antibody-dependent cell-mediated cytotoxicity (Goepfert 2002).

The author highlights the importance of another subset of the T-cells called the CD8+ cells. These CD8+ T cells are also referred to as cytotoxic T lymphocytes. According to the author, they target and lyse virally infected cells by recognizing foreign antigens bound by host proteins. Clearly, it can be seen that CD4+ T cells play a central role in the body's response to pathogens. HIV targets these cells, infects them and subsequently destroys them. This incapacitates the body`s defense system to any kind of infection (Goepfert 2002).

Both CD4+ and CD8+ T cells are very important in controlling HIV infection. A model of HIV immunopathogenesis based on virus-specific immunology has been hypothesized (Goepfert 2002). In this model, an individual is infected with HIV, and CD8+ T cells with the specific help of CD4+ T cells are supposedly able to efficiently neutralize the virus infecting the CD4+ T cells (Goepfert 2002).

According to DiPentima et al. (2005), HIV attacks specific cells of the immune system, takes over their protein making machinery, and multiplies. In other words, when infected with HIV, a T-cell becomes an HIV-replicating cell (Engenderhealth 2005). This process eventually causes the cell to die. As the number of CD4 cells decreases, the infected person's immune system becomes increasingly compromised (Engenderhealth 2005).

This subsequently destroys a substantial number of these cells, thus compromising the body's ability to fight off invading germs and disease (DiPentima et al. 2005). When the number of the cells of the immune system declines to a certain threshold, people infected with HIV become more susceptible to other infections that a healthy body would normally be able to fight off (DiPentima et al. 2005). The weakened immunity is known as AIDS and can result in severe life-threatening infections, some forms of cancer, and the deterioration of the nervous system (Engenderhealth 2005).

These diseases are called opportunistic infections because they take advantage of the weakened immune system. Examples of opportunistic infections include Crptococcal meningitis and Pneumocytis pneumonia.

HIV is transmitted through exchange of body fluids such as blood transmission, semen, vaginal secretions and breast milk (Stine 1993). Although AIDS is purported to be a result of an HIV infection, not everyone with HIV has AIDS. Notably, adults who become infected with HIV may appear healthy for a substantial number of years (usually when appropriate health advice is sought) before they get sick with AIDS (DiPentima et al. 2005).

When the body's defense system against foreign invasion fails to function properly, the condition is called AIDS (Acquired Immunodeficiency Syndrome) (Brown 1997). The virus known for causing this condition is called HIV (Human Immunodeficiency Virus). HIV is a retrovirus, a type of virus that stores its genetic information on a single-stranded RNA molecule. After a retrovirus penetrates a cell, it creates a DNA version of its genes, and its DNA becomes part of the infected cell's DNA (Engenderhealth 2005).

The first cases of AIDS related infections were diagnosed in 1981 in the United States of America and the infections were more pronounced among homosexual men than other individuals (Kaiser Family Foundation, 2007).

CHAPTER 2-LITERATURE REVIEW

2.1 Literature on patterns of and associations with immunologic response

2.1.1 Description of immunologic response

Immune response is basically described by the American Heritage Dictionary of the English Language (2003) as an integrated bodily response to an antigen, especially one mediated by lymphocytes and involving recognition of antigens by specific antibodies or previously sensitized lymphocytes. Several ART literature citations refer to immune response alongside virological response. Virological response describes HIV-1 RNA plasma levels below 50, 400 or 1000 copies/ml (Schechter and Tuboi, 2006). Some studies also refer to immunologic response in terms of CD4 cell count increases and/or CD4 cell count recovery or immunologic restoration (Lawn et al. 2006).

Most studies refer to an increase in CD4 cell count of at least 50 cells/μl (within a specified period) or maintenance of CD4 cell counts above a certain threshold (generally 200 cells/μl) as being representative of the beneficial immunologic response to ART (Battegay et al. 2006). Despite the use of the definition of immunologic response by some investigators as increase of at least 50 cells/μl, no clinical significance was attached to it (Loutfy et al. 2005). These authors evaluated this definition by Cox models in 228 ART patients infected with HIV with viral load > 1400 copies/ml. The 12 month CD4 cell count responses were divided into 5 categories, ranging from decrease or no change to greater than 100 CD4 cell/μl increase.

They reported that there was a lower risk of clinical progression for each incremental increase in CD4 cell count response. They also established that patients with 25 CD4 cell/μl increase were associated with a 21% reduction in the risk of an AIDS-defining event or death. This then implies that increases greater than 50 cells/μl were associated with even greater reductions in the risk of unfavorable clinical outcomes.

Meanwhile, studies report that achieving CD4 count over 200 cells/μl has been associated with reduced risk of opportunistic diseases and favorable clinical outcomes (Battegay et al. 2006). This is exemplified by recommendations to discontinue primary and secondary prophylaxis against a variety of pathogens when CD4 cell count has reached at least 200 cells/μl for 3-6 months (Battegay et al. 2006). These pathogens include *Pneumocystis jiroveci* and *Mycobacterium avium* complex (MAC) infections.

2.1.2 The effect of ART and the associated benefits

The hall mark of human immunodeficiency virus (HIV) infection is characterized by the continuous loss of CD4 T lymphocyte cells, leading to progressive immunodeficiency, opportunistic diseases, and death (Hirsch et al. 2004 and Battegay et al. 2006). Antiretroviral therapy results in an increase in the number of CD4 cells and the functional reconstitution of the immune system (Kelleher et al. 1996 and Autran et al. 1997). The expected outcome of treatment of patients infected with HIV with highly active antiretroviral therapy (HAART) is to suppress the HIV-1 RNA level in plasma and improve CD4 cell count response (Graber et al. 2000).

In those patients receiving highly active antiretroviral therapy (HAART) there is evidence of an initial rapid increase in CD4 count in the first three to six months after starting therapy, likely to be associated with redistribution of cells trapped in the lymphoid tissue (Battegay et al. 2006). The benefits of ART have been associated with major reductions in viral load and large reductions in morbidity and mortality from HIV infections (Smith et al. 2003; Schechter and Tuboi, 2006 and Tuboi et al. 2007).

However, an estimated 10-25% of patients starting ART at very low CD4 cell counts, usually below 50-100 cells per μl, may be affected by the immune reconstitution disease or immune reconstitution syndrome characterized by inflammatory reactions to previously asymptomatic pathogens (Battegay et al. 2006). The authors noted that most of these symptoms are manifested 2-8 weeks after the inception of ART. The mechanism of development of immune reconstitution syndrome is still not fully described (Battegay et al. 2006).

2.1.3 The ART effects on the immune response from onset of therapy

Many studies refer to immunologic response in terms of an increase of CD4 cell count beyond 50 cells/μl. This becomes more relevant when the length of period during which patients are on ART is considered as well (Smith et al. 2003; Lawn et al. 2006 and Battegay et al. 2006).

The rate of immune recovery measured by CD4 count, at two years after starting treatment, is slower than that at earlier times (Smith et al. 2003). For instance, one study by Kaufmann et al. (2002) found that the greatest increases in CD4 counts occur in the

first two years. However, the aforesaid study was reported as having limited statistical power and in addition not all of its participants were ART naïve. In a different study, Smith et al. (2003) also obtained convincingly consistent findings in which individuals who maintained virological suppression had an increase in CD4 count for at least three years after starting HAART.

A study by Lawn et al. (2006), which did not adjust for the viral load of the patients noted that within the first two years of ART, patients with baseline CD4 cell counts < 50 cells/μl had equivalent or greater capacity for immunological recovery during 48 weeks of ART compared to those with higher baseline CD4 cell counts. Despite higher rates of CD4 cell count gains associated with lower CD4 cell counts during the first year as suggested by Lawn et al. (2006), it has also been postulated that lower baseline CD4 cell count at initiation of antiretroviral therapy requires longer treatment periods to reach the desired CD4 cell count target level (Battegay et al. 2006).

As mentioned previously, most authors include the duration of treatment in explaining immunologic response. Attempts have been made to come up with predictive models to determine immunologic response at certain durations during the course of ART. For example, Lawn et al. (2006) reported that the period 0 to 16 weeks had the greatest rate of CD4 recovery and was strongly associated with baseline viral load. At the same time, patients with viral loads greater than 10^5 $\log_{10}$ copies/ml had greater CD4 count increases than those with lower viral loads. In the period 16 to 48 weeks, CD4 cell recovery was reported to be associated with younger age and baseline CD4. The authors reported that sustained suppression of viral load was critical at this stage. Lastly, at 16 weeks viral load

was also reported as a strong independent predictor of subsequent immunological recovery. From the above examples, it can be noted that at different phases, immunologic response is determined by different factors (Lawn et al. 2006).

A recent study in a high income setting by Mocroft et al. (2007) showed that CD4 cell count in patients with undetectable viral load can rise to levels similar to healthy individuals. The authors also noted that patients who start ART with baseline CD4 cell count above 350 cells/μl reach normal levels of CD4 cell count quicker than those with lower baseline CD4 cell count. The authors concluded by reporting the possibility of attaining normal CD4 cell counts in all patients regardless of their baseline CD4 cell count provided the viral load is kept below detectable levels.

Although most of patients in sub-Saharan Africa start ART with advanced disease (Lawn et al. 2006) the study by Mocroft and colleagues clearly demonstrate the possibility of immunological recovery in all patients.

2.1.4 Factors that possibly affect immune response

Most of the work done in immunologic response has included analysis that attempt to explain immunologic response in terms of several socio-demographic and clinical factors. Such factors have become variables which have been described in literature as predictors of immunologic response (Lawn et al. 2006). These factors have been modeled as independent variables in explaining immunologic response and tested for significance. For example, in one of these studies, Lawn et al. (2006) noted that the failure to attain 200 cells/μl was found to be associated with older age and lower baseline plasma viral load. In this way, older age would be a predictor of failure to attain 200 cells/μl.

In one Swiss study, Battegay et al. (2006) noted that not all patients receiving antiretroviral therapy attained complete recovery of CD4 count despite continuous suppression of plasma viral load. These findings perhaps suggested that there are several other factors which come into play other than excellently suppressed plasma viral load which determine complete recovery of CD4 cell count. These factors may for example include age, baseline CD4 cell count, WHO clinical category, sex and the plasma viral load (Lawn et al. 2006).

On the contrary, seemingly implicating poorly suppressed plasma viral load, Battegay et al. (2006) agreed with both Hansjee et al. (2004) and Douek et al. (2003). They all suggested that poorly suppressed HIV-1 replication is a major factor impeding the recovery of CD4 cells, leading to increased virus–related cell death and apoptosis. In their concluding remarks, Battegay et al. (2006) noted that entirely suppressed plasma HIV-1 RNA is not a definite prerequisite for an increase or stabilization of CD4 cell count.

HIV-1-infected patients with good virological responses and continuous plasma HIV-1 RNA levels below 1000 copies per mL, older age, long duration of HIV infection and lower CD4 cell count at baseline represented important risk factors for maintaining lower CD4 count (Kauffmann et al. 2002 and Kauffmann et al. 2005).

A study investigating the continued CD4 cell count increases in HIV-infected adults experiencing four years of viral suppression on antiretroviral therapy (Hunt et al. 2003) reported that the factors associated with increased CD4 cell count gains from three

months to four years included lower pre-therapy CD4 cell count, young age, female sex and infrequent lower-level viremia (versus sustained undetectable viremia).

The findings of the above study does not attempt to underplay the fact that gains in CD4 cell counts still occur in older age but at a slower rate than the younger age. This was illustrated by Douek et al. (1998). The increases which still occur in older age were said to be associated with increasing thymic output (Douek et al. 1998). In fact, the thymus is the site of the production and generation of lymphocyte T cells expressing ab-type T-cell antigen receptors (Douek et al. 1998). The authors indicate that the adult thymus can contribute to immune reconstitution following HAART. In conclusion, they highlighted that although the thymic function declines with age, a substantial output is maintained into late adulthood. In fact during the early days of HIV infection, thymic function decreases and that can be measured in the peripheral blood and lymphoid tissues (Douek et al. 1998).

This limits its activity to produce cells in the early days of HAART (Pido-lopez et al. 2003). As, HAART continues, the viral load particles decreases and the thymus recovers some of its functions to aid immune restoration (Pido-lopez et al. 2003). The increase in thymic function was reported by Douek et al. (1998) to be associated with increases in CD4 T cell numbers in HIV-1-positive patients during the course of HAART. This explains some of the gains in the CD4 cell count over time.

With reference to gender, the above findings were consistent with findings of another study by Giordano and colleagues. Giordano et al. (2003) followed up 100 virological

suppressed patients on ART for six months to determine whether CD4 cell count response to virus suppression during highly active antiretroviral therapy differs according to sex or race/ethnicity. Their findings were that women had greater CD4 cell count increases as compared to men and race/ethnicity was not a factor.

Explaining their findings, Giordano and colleagues reported that women repopulate their peripheral CD4 cells in response to virus suppression more quickly than men do. The authors suggested that women may have increased peripheral redistribution of memory CD4 cells from lymphoid tissue in response to viral suppression as a result of the effect of ART. To explain the long run gains of CD4 cell count increases which are more apparent in females than males, the authors also suggested that women may have more thymic output of naïve CD4 cells in response to HAART compared to men.

Also explaining the differences in CD4 response by gender, Giordano et al. (2003) cited pharmacokinetic differences between males and females probably also aligned to differences in physiological metabolic processes in the body. They suggested that these differences may result in women having higher antiretroviral drug levels, which subsequently lead to viral suppression and greater increases in CD4 cell count.

Gains in CD4 cell count were not surprisingly also associated with the AIDS clinical stage. The HAART-induced normalization of thymic output during the immune reconstitution results in larger increases in CD4 cell count in patients with more advanced disease, who had lower CD4 counts and lower numbers of naive T cells in particular

before treatment. In order to readapt towards normal cell levels, there is stimulation of production of more cells (Crossman 2005).

2.1.5 Immune response in Sub-Saharan Africa

In Sub-Saharan Africa, patients with access to antiretroviral therapy frequently have advanced immunodeficiency and consequently may have diminished capacity for CD4 count recovery (Lawn et al. 2006). Most individuals enrolled in ARV programmes in sub-Saharan Africa have median CD4 cell count below 100 cells/μl (Lawn et al. 2006). The authors also reported that such patients have been reported as attaining an elevated risk of morbidity and mortality before and after initiation of ARV therapy.

The potential for immune recovery has been suggested by Lawn et al. (2006) to be limited in patients with advanced pre-treatment immunodeficiency. They specifically noted that the capacity for restoration of CD4 cell counts and CD4 functional responses during ART has been reported to be associated with pre-treatment immunodeficiency. Seemingly contradicting these findings, Hunt et al. (2003) reported that the immune system's capacity for CD4 T lymphocyte restoration was not limited by low pre-therapy CD4 counts.

2.1.6 The effects of co-infections on the immune response

There has not been a definite conclusion on whether some co-infections such as HIV/hepatitis C virus (HCV) limit cell recovery whereas some appear to enhance increases in CD4 cell count such as HIV/ hepatitis G virus (HGB) (Battegay et al. 2006).

In one study by Lincoln et al. (2003) in Australia, it was reported that patients co-infected with HIV/HCV appeared to have a poorer response to HAART in terms of CD4 count changes, with a CD4 count increase of 32 cells/μl (95% CI 1–67) less than HIV-only patients. These investigators also found that co-infections with HBV or HCV were relatively common among the HIV-infected. In conclusion to these findings, they highlighted that outcomes following HAART did not appear to be adversely affected by HBV/HCV co-infection, except impaired CD4 count responses in HIV/HCV co-infected patients.

Another study by Atkinson et al. (2002), involving 103 individuals of whom 35% of the patients were co-infected with HCV and 86.1% of this proportion had acquired HIV by injecting drugs, found that HCV co-infection had no effect on CD4 response in antiretroviral-naïve patients during the first year of therapy. In their regressional analysis they found that there was no significant difference in the baseline, peak or rate of CD4 cell response, with respect to HCV serostatus. They highlighted that for those individuals with CD4 count of <200 cells/μl, the likelihood of having a CD4 count >200 cells/μl at 1 year was 51%. This demonstrates a high likelihood of favourable CD4 cell count response and minimal impairment of CD4 cell count compared to the already mentioned study by Lincoln et al. (2003).

2.1.7 Factors theoretically known to hinder complete immune response

There is also limited knowledge of the factors that determine CD4 cell responses and they seem to depend on both the host and the virus (Battegay et al. 2006). Theoretical factors known to impede complete recovery of CD4 count could possibly range from increased viral pathogenicity to certain inherent host factors (Battegay et al. 2006). In addition the authors also note that T-cell apoptosis and cell death may occur in patients with well

suppressed plasma HIV-1 Ribonucleic acid (RNA). They also suggest that such a scenario may further impede complete recovery of CD4 cell count.

One good example of such a case is seen in a Swiss study by Kaufmann et al. (2005). They found that 36% of the patients on ART did not reach CD4 cell counts above 500 cells/μl after 5 years despite continuous suppression of viral load to below 1000 copies/ml and almost half of these patients had reached a plateau in their CD4 cell count (Kaufmann et al. 2005).

2.1.8 Description of "Discordant or paradox" CD4 response

Population-based studies have demonstrated that in a large proportion of patients taking antiretroviral therapy their CD4 increases appear to occur without apparent significant reduction of viral load, an unusual behavior described as "discordant or paradox" CD4 response (Wood et al. 2002). The discordant CD4 response is not only associated with increases in CD4 cell counts but scenarios can also arise where patients on antiretroviral therapy seem not to exhibit significant rise in CD4 cell counts despite viral suppression (Tuboi et al. 2007).

Other examples of discordances include patients on ART for long periods that experience persistently low CD4 cell counts despite undetectable viral load. With regard to the aforesaid example, investigators at Chelsea and Westminster Hospital in London reviewed the records of over 1140 patients and found that after one year 16% failed to show an increase in CD4 cell counts above 50 cells/μl despite suppressed viral loads (Tung et al. 2005). Lastly, some patients may achieve undetectable viral load (falling viral load) on treatment but their CD4 cell counts continue to fall. For instance, one study

found 13% of over 300 people on treatment experienced substantial viral load reductions as well as falls in CD4 cell counts (Maggiolo et al. 1999).

2.1.9 Mechanisms of development of discordant CD4 cell count

There seems to be no definite explanation to the mechanisms underpinning the development of 'discordant' CD4 cell count response (Tuboi et al. 2007). The authors highlight that it is apparently dependent on the interaction of a multitude of viral and host factors. They linked possible explanations to the role of protease inhibitor-based responses related to decrease HIV-associated T-cell apoptosis by HIV protease inhibitor, changes in thymic output and possibly to the weakening of the protease inhibitor-resistant virus (Wood et al. 2002).

Making reference to findings of a closely followed cohort of ART naïve patients, Wood et al. (2002) highlighted that patients who exhibited discordant immunologic response in twelve months of ART had either a prior transient/gap period of presumably undetectable plasma viral load and/or partial suppression viral load to < 100 copies/ml. The same authors reported that these results suggested that partial viral suppression is the primary mechanism involved in discordant CD4 count increases.

2.1.10 Research done on discordant CD4 cell count

Graber et al. (2000) conducted a study on discordant outcomes involving 2,236 protease inhibitor (PI) naive patients in a developed country setting. Part of his findings were that 19% of the patients had significant CD4 cell count increases after six months of antiretroviral therapy in the absence of a significant viral load reduction. The authors had immunological success defined as an increase of CD4 cell count of more than 50 cells/μl

and virological success as a decrease in HIV RNA viral load of more than 1 log10 copies/ml, after 6 months on treatment.

These findings were consistent with other findings from several sources in which immunologic discordancy was reported to have occurred in 20 to 30% of patients 6 months to 2 years after starting HAART (Tuboi et al. 2007).

2.1.11 Research on discordant CD4 cell count response specific to low income nations

There are limited data on discordant responses in terms of the frequency and its associated prognostic significance in patients being treated in resource constrained countries (Schechter & Tuboi, 2006 and Tuboi et al. 2007). One first known study of discordancy done in a low income resource setting reported a finding of overall frequency of discordant (immunologic and virologic) response of 33.8% after six months of therapy (Tuboi et al. 2007). The findings of this study were comparing complete responders to immunologic, virologic and non-responders and also using the same definition as used above for immune response and virological response.

The Antiretroviral Therapy in Lower Income Countries Collaboration (ART-LINC), an epidemiological network of HIV/AIDS treatment programmes in Africa, Asia and South America reported on the frequency of discordant responses in 1916 previously treatment-naïve patients seen in 15 developing countries who initiated HAART between March 1996 and April 2004. A total of 269 patients (14%) were virological only responders and 365 (19%) were immunological only responders (Schechter et al. 2006). It can be noted

that the frequency of discordancy in low industrialized nations was reported to be almost similar to that of high income countries (Schechter & Tuboi, 2006).

2.1.12 Factors known to explain discordant response

As mentioned earlier on, there is scant information about the pathogenesis of discordant responses, which seems also to somehow depend on the interaction of a multitude of viral, host and treatment-related factors (Schechter & Tuboi, 2006). Blunted CD4 response despite suppression of viral replication has often been attributed to host characteristics, particularly older age (Schechter & Tuboi, 2006; Piketty et al. 2001; Moore et al. 2006). It has been hypothesized that the magnitude of immune restoration is dependent on thymus activity, which decreases with age (Schechter & Tuboi, 2006). Thus older age has been identified as a risk factor for immunologic discordancy.

Renaud et al. (1999) reported that slower rates of decrease in CD4+ cell counts before therapy initiation are associated with a slower early recovery, whereas sharper slopes of CD4+ cell decrease are associated with maximal subsequent cell distribution once therapy is instituted. This therefore implies that the rate of CD4 cell count depletion after HIV infection is established is also important (Tuboi et al. 2007).

Other factors that have been reported to be associated with immunologic discordancy include infection by HIV-2, drug toxicity, and the use of the combination tenofovir/didanosine (Schechter & Tuboi, 2006). The authors also highlighted that the use of zidovudine or didanosine as part of the antiretroviral regimen, the concurrent use of other myelotoxic drugs such as co-trimoxazole and the presence of certain co-

infections, such as HTLV-1, have all been associated with suboptimal CD4 cell count responses despite suppression of viral replication. Adherence to therapy may also influence the occurrence of discordant responses (Moore et al. 2006). In this study the authors reported that suboptimal adherence was found to be associated both with virological and immunological only responses.

Schechter & Tuboi, (2006) reported the absence of published controlled studies evaluating the impact of different antiretroviral regimens on the incidence of discordant responses. On the other hand, they also noted that most published observational studies include patients receiving protease inhibitor-based regimens. One observational study by Moore et al. (2006) reported the absence of association between regimen type and presence of discordant responses 3–9 months after HAART initiation.

2.1.13 The association of discordant response and risk of clinical progression

The majority of published studies have indicated that, in comparison with complete response, discordant responses are associated with an intermediate risk of death or clinical progression (Tuboi et al. 2007). The authors reported that discordant responses have been associated with increased risk of clinical progression and mortality in developed countries. Piketty et al. (2001), reported that in a 30 months follow up study, discordant responders at 12 months experienced significantly more AIDS-defining events than full responders.

A study by Nicastri et al. (2005) reported that immunologic responders (virological non-responders) and virologic responders (immunological non-responders) exhibited a significantly lower risk of clinical progression than non-responders but a 2.3 and 1.9-fold

greater risk of death or new AIDS-defining event than complete responders respectively. The study had a follow up period of 44 months involving 2100 antiretroviral experienced and naïve HIV patients. In light of these findings, it can be noted that discordant responses are associated with greater risk of clinical progression than concordant responders in both developed and low industrialized nations.

On the contrary, Wood et al. (2002) seemed to report absence of differences in progression to AIDS or death between discordant CD4 cell responders and concordant virologic/immunologic responders reported in population studies. Despite these contradictions, Tuboi et al. (2007) acknowledged that the frequency of risk factors of and for discordant response is the same for both high income and low income countries. Certainly, Gulick (2000) noted how discordant responses in HIV RNA level and CD4 cell counts are common. He agreed with Tuboi et al. (2007) by suggesting that patients with an immunologic response, with or without a virologic response, are at less risk for clinical progression than those without an immunologic response over 24 months.

The fact that Graber et al. 2000 found that the discordant response was maintained at 12 months in 56% of those with an immunologic response and 33.3% of those with a virologic response suggests that the combination of virologic and immunologic responses to therapy over time is likely to be the best predictor of clinical progression (Gulick 2000).

At present, there is limited work done in the Khayelitsha database investigating the patterns of immunologic response and to establish the clinical significance of patterns of

CD4 cell count response such as the discordant CD4 cell response beyond the first two years. The patterns and associations with CD4 response beyond the first two years and the clinical implications of discordant CD4 cell responses in naïve patients undergoing antiretroviral therapy in the context of the Khayelitsha programme remain largely unknown.

2.2 Rationale of the study

Since the inception of the MSF clinical database for patients accessing ART five years ago, no studies concerning CD4 response alone have been done. Specifically, the actual patterns of CD4 response and their associations over time have never been studied in the Khayelitsha cohort.

2.3 Justification

The justification for carrying out this research study is to help identify patterns of and associations with immunologic response and to subsequently establish the effect of time on CD4 cell count response. In some instances, CD4 cell counts fail to increase significantly despite well suppressed viral load reduction, a phenomenon known as "discordant" CD4 response (Wood et al. 2002). It is important to expand our knowledge of the patterns associated with immunological response in the Khayelitsha cohort as basis for identifying patients at risk of blunted responses. Data on the patterns of immunologic response in the cohort such as frequency of immunologic discordancy, the associated clinical outcomes, and long-term anticipated responses are important at a programme level and may help HIV/AIDS programmes anticipate future clinical challenges and service demands.

2.4 The study aim

The aims of this study are to

1. Describe patterns of and associations with immunologic CD4 response
2. Describe the associations with discordant immunologic CD4 response
3. Explore the outcomes of patients with discordant immunologic response

2.4.1 The specific objectives

The specific objectives of this study are:

1. To describe the patient population characteristics and HIV history

2. To describe CD4 response to ART in the Khayelitsha cohort over time

3. To explore baseline and time-varying associations with CD4 response

4. To explore the associations with CD4 response in patients over time who remained virologicaly suppressed

5. To describe the frequency of immunological discordant responses and determinants including the baseline CD4 cell count which includes

   a. describing the frequency at various durations on ART

   b. describing the determinants of this outcome

6. Explore the relationship between immunological response in the first year and the subsequent survival.

CHAPTER 3 - METHODS

3.1 The study design

The analysis of this study formed part of an existing prospective cohort study describing the outcomes of antiretroviral treatment of patients in Khayelitsha

3.2 The study population

The study population comprised adult patients accessing ART in Khayelitsha, Cape Town, South Africa. The majority of these patients in Khayelitsha lived in conditions of low socio-economic status and HIV transmission is predominantly heterosexual. They were started on ART between May 2001 and the end of 2005, and followed up until the end of 2006. Study subjects were all HIV positive and ART naïve and were 15 years and older

The first-line ART regimen was comprised of stavudine, lamivudine (3TC) plus a non-nucleoside reverse transcriptase inhibitor (efavirenz or nevirapine). The second-line regimen for those failing first line was comprised of lopinavir/ritonavir, zidovudine (AZT) and didanosine. Prior to 2004, some patients started on AZT/3TC. All patients with CD4 counts< 200 cells/μl received daily contrimoxazole prophylaxis.

3.3 Sample size and sampling

As mentioned earlier on, the analysis formed part of an existing prospective cohort study with more than 3000 HIV positive patients already in the database. Despite what may seem as a "large enough" sample size, it was also essential to determine the required sample size to ensure that the study was sufficiently statistically powered.

About 80% of patients were expected to have a 50 cells/μl change in CD4 counts between two and three years (Smith et al. 2003). Postulating that 75% of the patients would have a 50 cells/μl change in CD4 count during the same period, the sample size was determined. At 80% power to detect a difference between 75% and 80%, the sample size computation using Stata gave 357 patients as required for the analysis.

Also mentioned earlier on, immunologic discordant response occurred in about 20-30% of patients in low industrial nations. With a precision of 5%, the researchers wanted to be 95% confident that the proportion of immunologic discordancy lay in this range.

$$\text{At } 20\%: N=1.96^2 * 0.2 * 0.8/ (0.05^2) =246$$

Or

$$\text{At } 30\%: N=1.96^2 * 0.3*0.7/ (0.05^2) = 323$$

Therefore a sample size of about 350 patients was required for the subsequent analysis. The computed sample size was in fact smaller than the actual number of patients in the database. Thus, the number of patients to be used in the analysis exceeded the computed sample size.

No sampling was required since all patients in the database were used in the analysis.

3.4 Measurement and data collection

Trained personnel used standardized procedures to prospectively collect clinical, laboratory and treatment data from medical records. The blood CD4 cell count and viral load were collected at the same time points. The CD4 and plasma viral load levels were checked at the start of ART and then after every six months. The CD4 cell count and viral

load data were obtained up to about 28 days at either side of scheduled visit. Structured clinical and laboratory records were maintained on all patients screened on entry to the ART programme. During screening and follow-up, some of the captured information included demographic details (such as age, sex and place of residence), attendance at clinic, weight, diagnoses of infections, prophylaxis and treatment, CD4 cell counts and HIV RNA levels, sputum smears and culture results for TB, WHO clinical stage and antiretroviral treatment history.

3.5 Definitions of immunologic and virologic responses and clinical outcomes

Immunologic response was initially defined as an increase in CD4 cell count from baseline of more than 50 cells/μl. For the purposes of identifying patients with discordant immunologic response: these were patients who achieved virological suppression (<400 copies/ml) and at the same time failed to attain an increase of 50 cells/μl of CD4 cell count after 12 months of ART. AIDS defining events were defined according to the WHO staging system.

3.6 Data management, statistical analysis and inclusion and exclusion criteria

The data (both clinical and sociodemographic data) had already been entered in the database and this process was validated.

The sociodemographic and clinical description of patient population at baseline was done using descriptive measures (measures of central tendency & dispersion). These included means, medians, standard deviations, ranges and the inter-quartile range. Frequency distributions were used to summarize both continuous and categorical

variables. Thus, frequency description was based upon the analysis of the population continuous variables (such as age, HIV viral load, CD4 cell count) and categorical variables (such as gender and the WHO clinical stage). The HIV history was expressed as the period for which the patients had been HIV positive and the average period for which they had been on ART.

In order to show CD4 response to ART in the Khayelitsha cohort over time, graphs were used to show the frequency distribution of absolute blood CD4 cell counts at baseline, 6 months, 12 months, 24 months, 36 months, and 48 months. Blood CD4 cell counts response during ART was expressed as the mean or median change in CD4 cell count measured in cells/μl together with the corresponding standard deviation or inter-quartile range. The CD4 cell count changes were measured from baseline, at 6 monthly intervals until the 48th months. The rates of change were determined for all patients, followed by patients with suppressed viral load at successive time points and lastly in patients with suppressed viraemia throughout the follow up time until the given CD4 count measure.

Linear models, incorporating the population average estimator[†] were used to describe CD4 cell changes beyond six months stratified by time from the inception of ART to 48 months. The statistical method involved exploring the effect of time on CD4 response, taking into account repeated measurements. Time effects on CD4 cell count change were considered in individuals with baseline CD4 cell count less than 200 cells/μl in order to restrict the analysis to patients treated strictly according to protocol. The analysis was also adjusted for the effect of the initial regimen (nevirapine and AZT) on the CD4 cell

[†] It does not take into account the population distribution.

count. Discordant response was added as one of the exposure variables to establish its impact on CD4 cell count change after controlling for everything else.

As each person contributed more than one value to the model, the models are adjusted for intra-individual homogeneity. The autoregressive covariance matrix was imposed on the data to account for the increasing variability of CD4 cell count over time. On the other hand, the CD4 cell count changes at six months were assessed by simple linear regression.

The frequency of immunological discordance response was determined by subtracting the baseline CD4 cell count from the CD4 cell count after one year, after two years, after three and after four years. During each follow up point, the net increase in CD4 cell count was below 50 cells/μl and the plasma viral load was below 400 copies/ml. The determinants of discordant response in the first year of ART were assessed by logistic regression. The response variable was a binary variable describing those patients who failed to attain an increase of CD4 greater than 50 cells/μl whilst remaining virologically suppressed.

Survival analysis was used to access the relationship of discordant response in the first year and the subsequent survival beyond 12 months and the results were plotted on Kaplan Meier curve. The Cox model was then used to access the determinants of survival in patients with discordant response. Model diagnostics was used to identify influential observations and outliers in the models and no observations were identified to have a significant effect on the models. The level of significance was set at 0.05

Analyses were performed using STATA™ 9.0 Statistics/Data Analysis software package (Copyright 1984-2005, Stata Corporation, 4905 Lakeway Drive, College Station, Texas 77845 USA).

All patients in the database who were HIV positive and ART naïve were included in the analysis. Thus, each stage of the analysis included patients with adequate information for the subsequent computation.

3.7 Ethical and legal considerations

This study was part of a larger running MSF/UCT research project entitled "Enhanced Routine Surveillance of HIV clinic population in Khayelitsha". The ethics approval for this project was obtained from the Research Ethics committee of the Faculty of Health Sciences at the University of Cape Town.

The data for the analysis was extracted from the patient database for the same project. The names of patients were not included. Confidentiality of the patient details was maintained at all times throughout the analysis.

3.8 Reporting of the results

The results will be reported to all major stakeholders. These include University of Cape Town: Department of Public Health, Medecins Sans Frontières (MSF), and The Department of Health: Western Cape.

CHAPTER 4 - RESULTS

4.1 Descriptive analysis of the study

This section includes the description of the baseline characteristics of the patients undergoing community ART in Khayelitsha.

4.1.1 The population baseline characteristics

At the time of analysis, the total number of treatment naïve adult patients undergoing ART captured in the Khayelitsha study database was 3373, of which females and males constituted 69.9% (2358) and 30.1% (1015) respectively (Table 1). The median age was 32 years (IQR, 28-38). The median ages for males and females were 36 (IQR 31-41) and 31 (IQR 27-36) respectively. The sum rank test showed that there was a significant ($P \leq 0.001$) difference between the median ages of males and females. The age variable was categorized into four subgroups to facilitate subsequent analysis. These sub groups included patients with ages between 14-24 years, 25-34 years, 35-44 years and more than 44 years. The largest proportion of the patients (50.9%) was within the range 25-34 years.

The median baseline CD4 cell count was 87 cells/μl (IQR 35-146). Females had higher baseline CD4 cell counts than males ($P<0.001$) (Table 1). The proportion of patients with CD4 cell counts within the ranges <50, 50-99, 100-149 and greater than 150 cells/μl were 33.1%, 22.6%, 20.8% and 23.5% respectively. The median baseline $\log_{10}$ viral load was 5.11 copies/ml (IQR 4.59-5.57). At the time of initiation of HAART, 46.3% of the

patients had suffered from TB. Furthermore, 41.7% of the patients had AIDS defining illnesses.

Table 1. The baseline characteristics of the patients included in the analysis

Characteristics	N (%)	Median (IQR)	P-value
Age (years)			
All[1]	3373 (100%)	32 (28-38)	
Females	2358 (69.9%)	31(27-36)	
Males	1015 (30.1%)	36 (31-41)	P<0.01[2]
14-24	345 (10.2%)		
25-34	1717 (50.9%)		
35-44	980 (29.1%)		
≥44	331(9.81%)		
Baseline CD4 cell count (cells/μl)			
All	3282 (97.3%)	87 (35-146)	
Males	989 (29.2%)	74 (30-139)	
Females	2293 (70.8%)	92 (39-149)	P<0.01[3]
≤49	1085 (33.1%)		
50-99	743 (22.6%)		
100-149	683 (20.1%)		
≥150	771 (23.4%)		
<200	3106 (94.6%)		
≥200	176 (5.36%)		
Baseline log10 viral load			
All	2945 (87.3%)	5.11 (4.59-5.57)	
0-4	230 (7.81%)	3.65 (1.99-4.00)	
4.1-5	1071 (36.4%)	4.6 (4.34-4.85)	
5.1-6	1403(47.6%)	5.44 (5.00-6.00)	
>6	241(8.81%)	6.25 (6.04-6.72)	
Prior TB[4]			
All	3373 (100%)		
Yes	1574 (46.7%)		
No	1799 (53.3%)		
AIDS defining illness			
All	3373 (100%)		
Yes	1406 (41.7%)		
No	1967 (58.3%)		
Year of diagnosis			
All	3291 (97.6%)		
Before 2000	274 (8.33%)		
2000-2002	1137 (34.6%)		
2003-2004	1361 (41.4%)		
2005-2006	519 (15.8%)		
Period under HAART (days)			
All	3373 (100%)	529 (339-529)	

[1] The percentage in brackets denoted by "All" refers to the proportion of the total cohort of 3373 patients for whom data was available. Subsequent percentages refer to the subset of patients with data for the given parameter.

[2] Wilcoxon sum rank non parametric test on the equality of median age between males and females.

[3] Wilcoxon sum rank non parametric test on the equality of median baseline CD4 cell count between males and females.

[4] Prior TB refers to history of TB infection before start of ART. Data on current TB infection was not available.

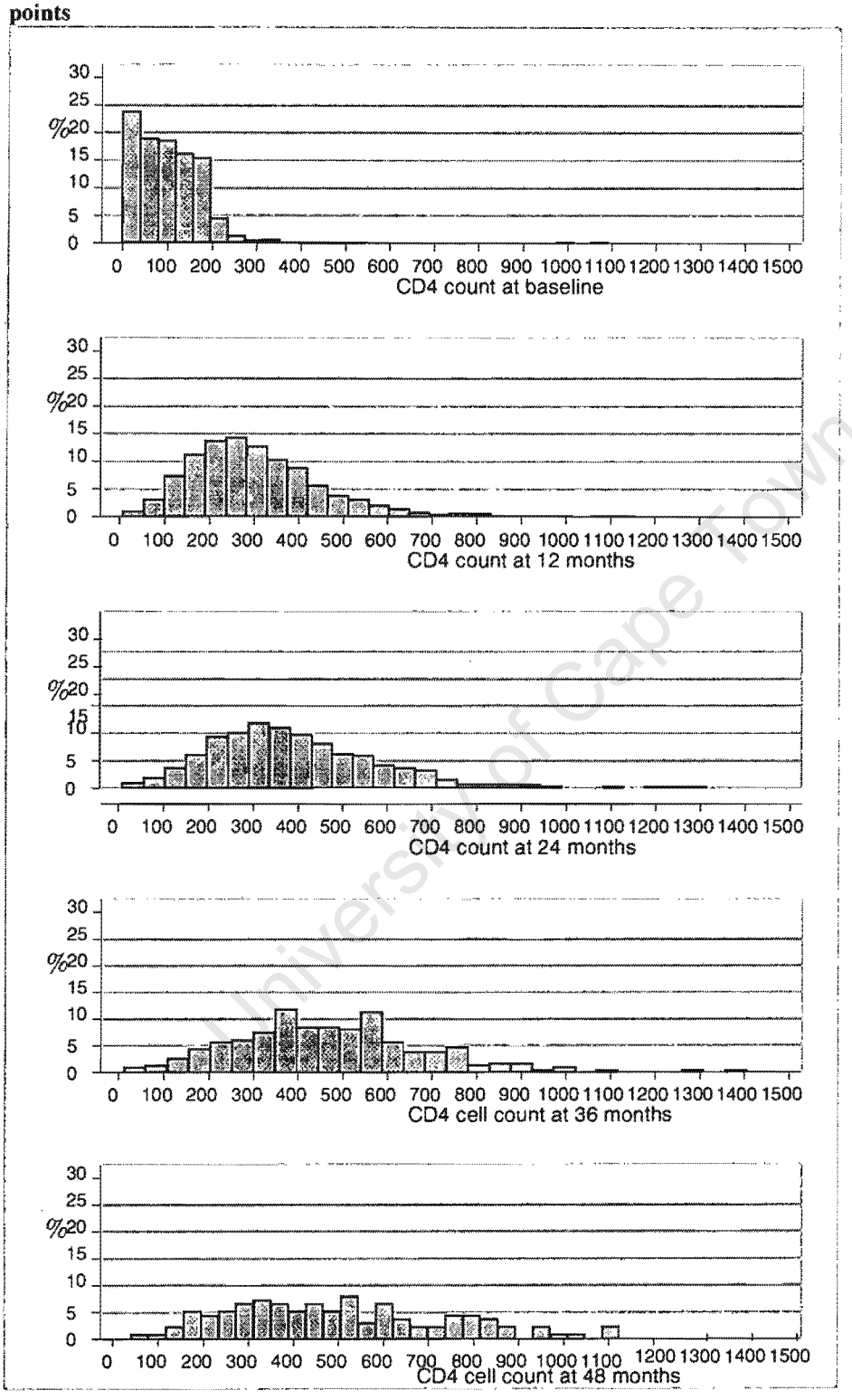
Most of the patients were diagnosed between years 2003 and 2004 (41.4%) whereas the smallest proportion of patients was diagnosed before year 2000 (8.33%). The median period for which the patients were on HAART by the time of the analysis was 529 days (IQR 339-529).

Van Cutsem et al. (2007) conducted a secondary analysis of the Khayelitsha HIV programme. They used the same data which our study analyzed. Their analysis reported that 59% of the patients started ART within two years of testing HIV. At the time of initiation of ART a third of the patients were reported to be on TB treatment. They used survival analysis to determine the loss to follow up and mortality. From this analysis, 16.9% (95% CI 13.6 – 21.0) of the patients were dead after 5 years of follow up. They defined loss to follow up as a year without a visit. Using this definition, they reported a loss to follow up of 20.3% (95% CI 16.9 – 24.3), this proportion also includes patients who died. By 5 years, a cumulative proportion of 11.0% (95% CI 8.2 – 14.7) had switched to second line. Of the patients who had been on treatment for 5 full years, 7/34 (20.6%) were on second line.

4.2 Description of the CD4 response to ART over time

This section includes the description of absolute CD4 cell response together with corresponding rates of increase at follow up time points of every six or twelve months during the 48 months of ART.

Figure 2: The frequency distribution of absolute blood CD4 cell counts at each of the follow-up time points



n=3282

n=2151

n=934

n=329

n=141

Table 2 Changes in blood CD4 cell counts and plasma viral load during ART

	Baseline	6 months	12 months	18 months	24 months	30 months	36 months	42 months	48 months
Virological response									
No. of patients		2260	2142	1431	931	526	339	190	139
No. (%) of patients with VL<400		2457 (92.4)	1894 (88.4)	1234 (86.2)	799 (85.8)	458 (87.1)	300 (88.4)	163(85.8)	122 (87.7)
Absolute CD4 cell counts									
No. of patients (All)	3282	2645	2151	1409	934	510	329	190	141
Median (IQR) CD4 cell Count (cells/μl)	87 (35-146)	233 (163-313)	282 (200-380)	323 (227-435)	369 (259-496)	405 (290-552)	457 (327-582)	435 (314-622)	476 (314-656)
CD4 cell count change									
No. of patients[1]		2579	1977	1298	834	455	277	162	110
Median (IQR) CD4 cell count change (cells/μl) over six monthly intervals		135 (79-201)	52 (-1-109)	48 (-8-111)	49 (-9-111)	39 (-18-117)	36 (-46-110)	23 (-59-100)	42 (-37-125)
No. of patients (%) (Virologicaly suppressed at two time points[2])		2272 (88)	1579 (80)	1013 (78)	636 (76)	357 (78)	225 (81)	125 (77)	86 (78)
Median (IQR) CD4 cell count change (cells/μl) over six monthly intervals		137 (81-206)	56 (7-14)	54 (1-121)	54 (1-118)	46 (-13-121)	42.5 (-45-114)	30 (-54-110)	43 (-46-149)
No. of patients (%) (Virologicaly suppressed throughout the follow up period)		53	50	45	48	51	49	47	48
Median (IQR) CD4 cell count change (cells/μl) over six monthly intervals		149 (103-225)	69.5 (10-131)	45 (-32-97)	71.5 (12-128)	53 (-32-106)	17 (-48-75)	6 (-68-88)	79 (-48-175)

[1]The number of patients refers to patients with available CD4 count data at two consecutive time points. Thus No. of patients at 6 months refers to patients with CD4 cell count data at both baseline and at six months and so on.

[2]These are successive time points, for example CD4 cell count change at 18 months would refer only to viral suppression at 18 and 12 months.

4.2.1 The description of absolute blood CD4 cell count over time

Graphs in Figure 1 show the frequency distribution of absolute blood CD4 cell counts at each of the follow-up time points that is at baseline, 12 months, 24 months, 36 months and 48 months, demonstrating not only increasing absolute CD4 count over time, but also increasing dispersion of values.

The virological response to ART was generally good as this was reflected by the fact that viral load was suppressed to less than 400 copies/ml in more than 86% of the patients at each of the follow-up time points (Table 2) after one year of treatment.

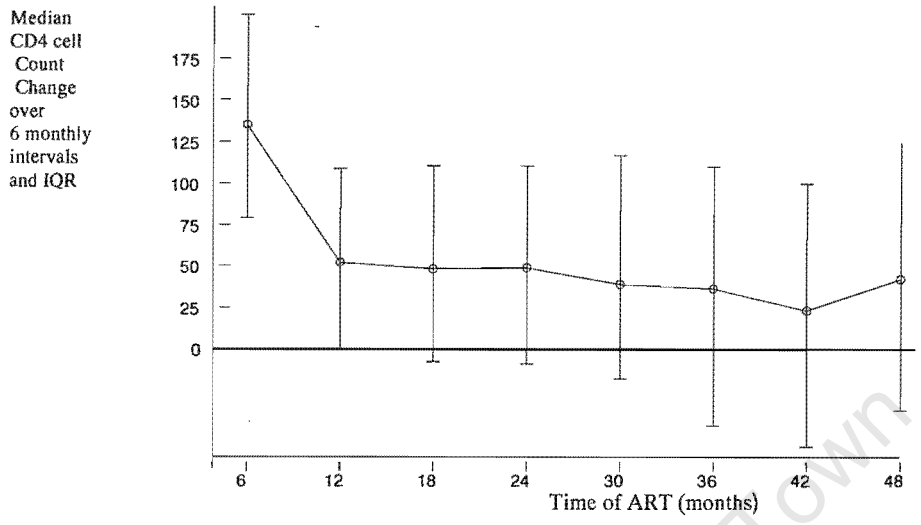
Although the proportion of patients with CD4 cell counts less than 200 cells/μl was 94.2% at baseline (Fig 1), this proportion decreased to 25.1% after 12 months of treatment. After 24 months, 36 months and 48 months of ART this proportion further decreased to 12.4%, 8.10% and 8.62% respectively (Fig 1). The median blood CD4 cell count increased threefold from a level of 93 cells/μl (IQR 41-149) at baseline to 281 cells/μl (IQR 199-379) after one year of ART therapy (Table 2). The median absolute CD4 cell count continued to rise throughout the four years of follow-up.

4.2.2 The rates of CD4 count increases at each of the follow up time points

The rate of the CD4 cell count increase in the first six months exceeded the rates of the preceding follow up time points at 12, 18, 24, 30, 36, 42 and 48 months (Table 2, fig 3). The same trend applied to the 12 months intervals rates which were characterized by higher rates in the first year than the subsequent years[‡]. However, when comparing the aforementioned follow up time points at 12, 18, 24, 30, 36, 42 and 48 months, the rates of increase of CD4 cell count were almost similar.

[‡] Results not shown

Figure 3: CD4 cell count slope during 48 months of ART



No. of patients	2579	1977	1298	834	455	277	162	110

The number of patients refers to patients with available CD4 count data at two consecutive time points. Thus No. of patients at 6 months refers to patients with CD4 cell count data at both baseline and at six months and so on.

The pattern of CD4 cell count increases was divided into two phases. The first phase comprised the fast/rapid phase of the blood CD4 cell count increase (that is, the first six months; median=135 cells/μl over six months (IQR 79-201)) and the second phase, comprising follow up times at 12, 18, 24, 30, 36, 42 and 48 months was mainly constituted by a slower phase-[§]median= 41.1 cells/μl per six months period.

4.3 The baseline and time varying associations of CD4 response

The analysis for this section included describing the effect on baseline CD4 cell count on CD4 cell change and this was done by stratification. This was then followed by another analysis composed of four models. The first model focused on the CD4 cell count

[§] The average of the median changes at 12, 18, 24, 30, 36, 42 and 48 months

changes at the first six months of ART. The other three models assessed the baseline and the time varying associations of CD4 cell count beyond six months.

These last three models focused on CD4 cell count change beyond the first six months during the 48 month follow up time. Each of these three different models included 1) all patients, without any regard to their viraemia status, 2) patients with suppressed viraemia at any two consecutive time points and 3) patients with suppressed viral load throughout the 48 months follow up time.

The first 6 months period had a separate model because the baseline characteristics were highly associated with higher differences in CD4 cell change in the first six months. Since the strength of intra-individual association weakens as CD4 cell count measurements are taken at longer durations on ART, the autoregressive covariance matrix was used. Each patient has more than one CD4 cell count measurement from different time points included in the analysis. The analysis makes adjustments for repeated measurements of CD4 cell counts. As each person could contribute more than one value to the model, the models were thus adjusted for intra-individual homogeneity. The final analysis was also adjusted for the previous CD4 cell count category.

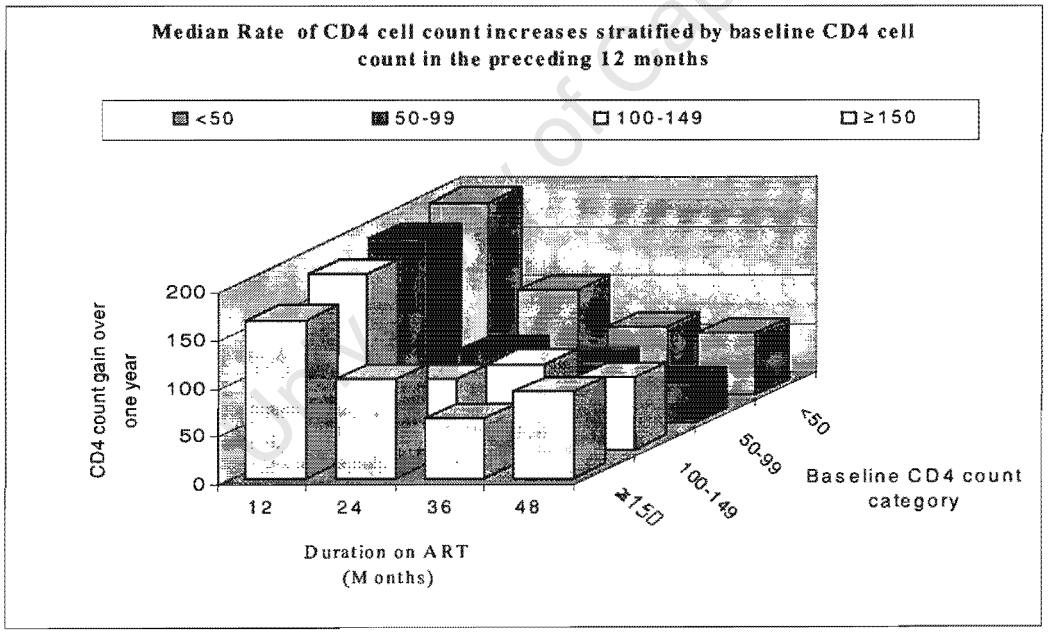
4.3.1 The effect of baseline CD4 cell count on the rates of CD4 increases

When stratified by baseline CD4 cell count, the rates of CD4 cell increases were the highest at 12 months follow up point in the baseline category <50 cells/μl compared to higher baseline categories (P<0.01). However, this increase declined over time. (Table 3, fig 3). After 36 months, patients with higher baseline CD4 cell counts started to have significant gains in CD4 cell count.

Table 3. The median CD4 cell count increase in the preceding 12 months stratified by baseline CD4

Baseline CD4 count (cells/μl)		<50	50-99	100-149	≥150
12 months					
	Number	638	507	463	493
	Median Rate IQR (cells/μl/year)	197 (128-290)	189 (117-279)	182 (109-257)	165 (85-272)
24 months					
	Number	319	210	174	157
	Median Rate IQR (cells/μl/year)	108 (40-188)	75 (15-181)	75 (14-172)	104 (20-191)
36 months					
	Number	133	67	56	42
	Median Rate IQR (cells/μl/year)	70 (8-159)	63 (-10-146)	90 (-6- 179)	63 (-81-129)
48 months					
	Number	62	25	25	12
	Median Rate IQR (cells/μl/year)	64 (-13-122)	41 (3-117)	76 (-28-193)	91 (46-123)

Figure 4: CD4 count changes stratified by baseline CD4 cell count



4.3.2 Associations with CD4 cell count change at the first six months of ART in all patients

Several factors were independently associated with CD4 cell count changes after six months of therapy (Table 4). These factors included age, sex, baseline CD4 cell count,

suppressed viral load at six months count and log10 baseline viral load. On the other hand, CD4 cell count change in the same period was not associated with clinical stage and prior TB.

Notably, changes in CD4 cell count at six months decreased with increasing age. For instance, patients older than 44 years gained 35.5 fewer CD4 cells/μl than patients between 14 and 24 years (95% CI-56.7;-14.4). Males gained 20.6 fewer CD4 cells/μl than females (P<0.01; 95% CI -31.0; -10.3) after six months of therapy.

At six months, gains in CD4 cell counts decreased with increasing baseline CD4 cell count relative to the lowest baseline CD4 cell category (Table 4). For example, patients with baseline CD4 cell count greater than 150 cells/μl had 14.3 fewer CD4 cells/μl (95% CI -27.6; -1.3) compared to those who had less than 50 cells/μl at baseline (Table 4). Every unit increase in the log 10 of baseline viral load was associated with a gain of 14.5 CD4 cells/μl (P<0.01). Evidence of good virological suppression at six months was associated with gains in CD4 cell count (Table 4). Suppression of viral load at six months was associated with a gain of 25.8 more CD4 cells/μl than those patients who were not suppressed.

Table 4 Prediction of CD4 cell change at the first six months of ART in all patients**

Variable		Mean change (cells/μl)	P value	95% CI	
Age					
	14-24	Reference group			
	25-34	-10.4	P<0.01[a]	-26.7	5.8
	35-44	-17.6		-34.8	-0.5
	≥44	-35.5		-56.7	-14.4
Sex					
	Females	Reference group			
	Males	-20.6	P<0.01	-31.0	-10.3
Clinical stage					
	Stage 1, 2 & 3[b]	Reference group			
	Stage 4	2.6	0.6	-7.3	12.4
Baseline CD4 cell count (cells/μl)					
	0-49	Reference group			
	50-99	-2.4		-15.2	10.5
	100-149	-7.3	P<0.01[a]	-20.5	5.9
	≥150	-14.5		-27.6	-1.3
Baseline viral load per one log increase		14.5	P<0.01	8.1	20.9
Prior TB[c]					
	No	Reference group			
	Yes	3.0	0.5	-6.3	12.4
Viral load suppression at six months					
	No	Reference group			
	Yes	25.8	P<0.01	11.2	40.4

[a] Wald test computation which gave a combined P-value for the following variables: Age groups and Baseline CD4 cell count. The test prevents Type 1 error which can occurs when comparing across catergories within groups.

[b] The non AIDS clinical stage was comprised of stages 1, 2 & 3. Stage 4 was the AIDS clinical stage.

[c] The prior TB variable refered to the history of TB infection before inception of ART.

4.3.3 The prediction of CD4 cell change beyond six months of ART

The following three models describe the patterns of CD4 cell count changes during the 48 months of treatment excluding the first six months (Table 5, 6 and 7). Amongst the three models, as mentioned earlier on, the first model focused on describing the baseline and

** Predicted change in CD4 cell count changes was assessed using multivariable linear regression. The computation included 1961 patients. All listed predictors were included in the multivariable model, which had an R^2=0.089.

time varying association of CD4 cell count change in all patients, the second model analysed the baseline and time varying association of CD4 cell response patients among patients with suppressed viraemia at any two consecutive time points. Lastly, the third model described the baseline and time varying association of CD4 cell response in all patients with continuously suppressed viral load up until the time of each measurement.

All three models exhibited similar patterns in terms of baseline and time varying CD4 cell change (Table 5, 6 and 7). Of the three models, the first model which focused on the time varying associations of CD4 cell count beyond six months in all patients reported that CD4 cell change was associated with age, sex, last CD4 cell count category, baseline viral load, continuously suppressed viral load and baseline CD4 cell count (Table 5). However, CD4 cell change was not associated with follow up time points, clinical stage and prior TB.

In this same model, the gains in CD4 cell count decreased with increasing age. Patients of the age group greater than 44 years gained 15.2 fewer CD4 cells/μl than patients between the ages of 14-24 years (Table 5) ($P<0.01$).

Males gained 15.8 fewer CD4 cells/μl than females. The gains in CD4 cell count over time increased with increasing baseline CD4 count. It could be noted that relative to patients with baseline CD4 cell count between 0-49 cells/μl, the CD4 count gains increased from 13.1 fewer CD4 cells/μl in the category between 50-90 cells/μl to 1.5 more CD4 cells/μl in patients with baseline CD4 cell count over 150 cells/μl ($P<0.01$) (Table 5). Higher previous CD4 cell count categories were associated with less CD4 cell

count gain. For example, patients with previous CD4 cell count greater than 500 cells/μl were associated with 36.7 fewer CD4 cells/μl than those between 200 and 349 cells/μl (P<0.01).

It was also interesting that more CD4 cells were gained from continuously suppressed viral load as compared to gains associated with baseline viral load. This suggested that over time, continuously suppressed viral load also becomes a major determinant of CD4 cell count gain. Conversely, previously suppressed viral load was not associated with CD4 cell count change over time[††]. Those with continuous suppression of viral load had a gain of 22.1 more CD4 cells/μl than those without any suppression (Table 5).

In contrast to the model without restriction to viraemia status, the model with patients who suppressed viraemia at any two consecutive time points had higher differences in gains in CD4 cell count by age (Table 5 and 6) (P<0.02). Notably, patients older than 44 years gained 22.3 fewer CD4 cells/μl than patients between the ages of 14 and 24.

[††] Results not shown

Table 5 Prediction of CD4 cell count change beyond 6 months of ART in all patients‡‡

Variable		Mean change (cells/µl)	P value	95% CI	
Age					
	14-24	Reference group			
	25-34	2.1	P<0.01[a]	-8.7	13.0
	35-44	-7.5		-19.1	4.1
	≥44	-15.2		-30.0	-0.4
Sex					
	Females	Reference group			
	Males	-15.8	P<0.01	-23.0	-8.6
Clinical stage					
	Stages 1, 2 & 3[b]	Reference group			
	Stage 4	6.1	0.1	11.8	32.3
Baseline CD4 cell count					
	0-49	Reference group			
	50-99	-13.1		-21.3	-4.9
	100-149	-10.9	P<0.01[a]	-19.7	-2.1
	≥150	1.5		-9.1	12.1
Time					
	12 months	Reference group			
	24 months	-3.7		-21.7	14.4
	36 months	6.0	P<0.16[a]	-10.7	22.6
	48 months	-2.6		-21.0	15.8
Last CD4 cell count category					
	200-349	Reference			
	≤49	30.8		-7.9	69.4
	50-199	3.6	P<0.01[a]	-5.5	12.7
	350-499	-11.3		-20.8	-1.8
	≥500	-35.3		-48.0	-22.6
Baseline viral load per one log		4.5	P<0.01	0.3	8.6
Prior TB[c]					
	No	Reference group			
	Yes	-6.12	0.1	-12.45	0.21
Continuously suppressed viral load					
	No	Reference group			
	Yes	22.1	P<0.01	11.8	32.3

[a] Wald test computation which gave a combined P-value for the following variables: Age groups, Last CD4 cell count category, Baseline CD4 cell count & Time. The test prevents Type 1 error which can occur when comparing across catergories within groups.

[b] The non AIDS clinical stage comprised stages 1, 2 & 3. Stage 4 was the AIDS clinical stage.

[c] The prior TB variable referred to the history of TB infection before inception of ART.

‡‡ Predicted CD4 cell count change was assessed using a multivariate cross-sectional time-series regression model which incorporated a population average estimator which also is the same as a random-effects estimator in linear regression. The computation included 2933 observations from 946 patients. The average observation per group was 3.2 (range 2-7). During the computation, observations were not equally spaced and thus 99 patients were omitted from the estimation. Some patients had fewer than 2 observations, thus it was not possible to estimate correlations for those groups and consequently 660 patients were omitted.

Furthermore, clinical stage became an important predictor of CD4 cell count change in the model which included patients with successive suppressed viraemia, whereas it was not in the model which included all patients. With respect to the other variables, except for age, there were similar differences in CD4 cell count changes over time between the model where there was no restriction by viraemia status and the model where patients had suppressed viraemia at any two consecutive time points (Table 5 and 6). The CD4 cell count changes were approximately similar in both models by variables such as sex, and baseline CD4 cell count ($P<0.05$).

When the analysis was restricted to patients with successive virological suppression, the model indicated that males gained 17.7 fewer CD4 cells/μl than females. Those with AIDS clinical stage gained 8.2 more CD4 cells/μl than those with no AIDS clinical stage ($P=0.03$). Gains in CD4 count increased with increasing baseline CD4 cell count ($P<0.01$). Every unit increase in log 10 baseline viral load was associated with a gain of 5.4 CD4 cells/μl. The variables time, prior TB and continuously suppressed viral load were not important predictors of CD4 cell change over time in patients with successive suppressed viral load.

In the model in which the viral load was suppressed throughout, prior TB history and follow up time were the only variables not associated with CD4 cell count change over time (Table 7).

Table 6 Prediction of CD4 cell count change beyond 6 months of ART in patients with successive suppressed viral load†

Variable		Mean change (cells/µl)	P value	95% CI	
Age					
	14-24	Reference group			
	25-34	-3.1	0.02[a]	-15.5	9.4
	35-44	-9.5		-22.6	3.6
	≥44	-22.3		-38.9	-5.6
Sex					
	Females	Reference group			
	Males	-17.7	P<0.01	-25.8	-9.6
Clinical stage					
	Stage 1, 2 & 3[b]	Reference group			
	Stage 4	8.2	0.03	0.9	15.6
Baseline CD4 cell count (cells/µl)					
	0-49	Reference group			
	50-99	-12.8		-22.3	-3.8
	100-149	-8.4	P<0.01[a]	-18.6	0.7
	≥150	4.5		-12.6	11.5
Time					
	12 months	Reference			
	24 months	5.6		-14.8	26.0
	36 months	12.3	0.2[a]	-6.4	30.9
	48 months	1.0		-18.6	20.6
Last CD4 cell count category					
	200-349	Reference			
	≤49	10.7		-38.8	60.2
	50-199	0.8	P<0.01[a]	-9.5	11.2
	350-499	-9.5		-19.9	0.9
	≥500	-36.7		-50.2	-23.1
Baseline viral load per one log increase		5.4	0.02	0.9	9.9
Prior TB[c]					
	No	Reference group			
	Yes	-3.1	0.4	-10.1	4.0
Continuously suppressed viral load					
	No	Reference group			
	Yes	-3.8	0.6	-18.2	10.5

[a] Wald test computation which gave a combined P-value for the following variables: Age groups, Last CD4 cell count category, Baseline CD4 cell count & Time. The test prevents Type 1 errors when comparing across catergories within groups.

[b] The non AIDS clinical stage was comprised of stages 1, 2 & 3. Stage 4 was the AIDS clinical stage.

[c] The prior TB variable refered to the history of TB infection before inception of ART.

†Predicted CD4 cell count change was assessed using a multivariate cross-sectional time-series regression model which incorporated population average estimator which also is the same as random-effects estimator in linear regression. The computation included 2394observations from 826 patients. The average observation per group was 3.1 range (2-7). During the computation, observations were not equally spaced and thus 62 patients were omitted from the estimation. Some patients had fewer than 2 observations thus it was not possible to estimate correlations for those groups and consequently 626 patients omitted. The previous viral load variable was dropped due to collinearity.

Table 7 Prediction of CD4 cell count change beyond 6 months in all patients with continuously suppressed viral load[§§]

Variable		Mean change (cells/μl)	P value	95% CI	
Age					
	14-24	Reference group			
	25-34	-2.2	P<0.01[a]	-14.9	10.6
	35-44	-8.0		-21.3	5.2
	≥44	-23.3		-40.1	-6.6
Sex					
	Females	Reference group			
	Males	-19.1	P<0.01	-27.4	-10.9
Clinical stage					
	Stages 1, 2 & 3[b]	Reference group			
	Stage 4	8.6	0.02	1.1	16.1
Baseline CD4 cell count (cells/μl)					
	0-49	Reference group			
	50-99	-16.9		-26.4	-7.4
	100-149	-12.4	P<0.01[a]	-22.3	-2.6
	≥150	-3.7		-16.0	8.5
Time					
	12 months	Reference group			
	24 months	4.9		-17.3	27.1
	36 months	10.6	0.3[a]	-10.0	31.2
	48 months	-1.3		-23.0	20.4
Last CD4 cell count category					
	200-349	Reference group			
	≤49	5.7		-42.2	53.7
	50-199	0.5	P<0.01[a]	-10.0	11.0
	350-499	-10.7		-21.4	0.0
	≥500	-43.6		-57.8	-29.4
Baseline viral load per one log increase		5.5	0.02	0.9	10.1
Prior TB[c]					
	No	Reference group			
	Yes	-5.6	0.1	-12.9	1.6

[a] Wald test computation which gave a combined P-value for the following variables: Age groups, Last CD4 cell count category, Baseline CD4 cell count & Time. The test prevents Type 1 error which can occur when comparing across catergories within groups.
[b] The non AIDS clinical stage was comprised of stages 1, 2 & 3. Stage 4 was the AIDS clinical stage.
[c] The prior TB variable refered to the history of TB infection before inception of ART.

[§§] Predicted CD4 cell count change was assessed using a multivariate cross-sectional time-series regression model which incorporated a population average estimator which also is the same as a random-effects estimator in linear regression. The computation included 2287 observations from 786 patients. The average observation per group was 3.1 range (2-7). During the computation, observations were not equally spaced and thus 13 patients were omitted from the estimation. Some patients had fewer than 2 observations thus it was not possible to estimate correlations for those groups and consequently 585 patients were omitted.

The variables sex, age, clinical stage, last CD4 count and baseline CD4 cell count and viral load had approximately the same effect on changes of CD4 cell count over time as observed in the other models above (Table 5, 6 and 7).

In summary, in all of the three models[***], the time varying CD4 cell count changes were all commonly associated with age, sex, last CD4 cell count category and baseline CD4 cell count and log 10 baseline viral load. The CD4 cell gain decreased with increasing baseline CD4 cell count, except in those with baseline CD4 counts above 150 cells/μl. The CD4 count increased with log increase in baseline viral load. Gains in CD4 cell count decreased with increasing age over time. Where suppression is present, be it at any two time points or throughout the follow up period, the baseline clinical stage was an important predictor of CD4 cell count change over time. In the model for predicting CD4 change beyond six months in all patients, continuously suppressed viral load was also associated with CD4 cell change over time but last suppressed[†††] viral load showed no association. In all the models, follow up time and prior TB were not associated with CD4 cell count change. The initial regimen had no effect on CD4 cell count change at six months and beyond six months. When the discordant status was added into the model as exposure variable, it did not affect subsequent gains after controlling for everything else.

4.4 The patterns of and associations with discordant immune response during ART

This section describes the frequency of discordant immune responses at each follow up time measured from the inception of ART and its possible determinants. The risk of

[***] The first model included all patients, without any regard to their viraemia status, second model included patients with suppressed viraemia at any two consecutive time points and third model include patients with suppressed viral load through out the 48 months follow up time point

[†††] Results shown

discordant CD4 cell count response in patients with continuously suppressed viraemia was also estimated. Next, the subsequent survival beyond 12 months and its associated determinants by discordance response were determined. As defined earlier on, discordant immune response referred to patients who attained less than 50 cells/μl increase of CD4 cell count from baseline despite suppressing viral load to levels below 400 copies/ml.

4.4.1 The frequency of discordant response in the cohort

The frequency of immunologic discordant responses is been shown in Table 8 below.

Table 8 Frequency of discordant response at each follow up time point

	Six months	12 months	24 months	36 months	48 months
Total number of patients	2281	2367	2367	2373	_
Number[¥] (%) with discordant	283 (11.9)	99 (4.16)	13 (0.55)	1 (0.04)	_

[¥]The Discordant response was determined from difference of the baseline CD4 cell count and the CD4 cell count at each follow up time point among patients who were virologically suppressed. Patients with discordant response would be those with less than 50 cells/μl increase in CD4 cell count

The proportion of patients with discordant response was the highest (8.19 %) in the first six months of ART (Table 8). There was a reduction in this proportion in the second six months. There was a further decline after 24 and 36 months. There were no cases of discordant response at 48 months.

4.4.2 The risk factors of discordant immune response in the first year of ART

The determinants of the risk of immunologic discordant response were identified by multivariate logistic regression and the results are shown in Table 9 below. The analysis included patients with suppressed viraemia for the first twelve months of therapy.

Age was a risk factor independently associated with discordant response. In fact, the risk of immunologic discordant response increased with increasing age ($P<0.01$). For example, the patients in the age range greater than 44 years old had approximately five fold increased risk of having a discordant response compared to patients in the age range of 14- 24 years old (Table 9). The proportion of the patients with age between 14-24, 25-34, 35-44 and >44 who failed to attain an increase of greater than 50 cells/μl despite suppressed viremia were 5.13%, 4.70%, 7.11%, and 11.0%.

Baseline CD4 cell count was also a risk factor independently associated with discordant response. There was also a clear increasing risk with increasing baseline viral load ($P<0.01$). Thus, high baseline CD4 cell count was associated with the greatest risk of discordant response in the first year of ART. Patients with lower baseline CD4 cell count had a lower risk of discordant response compared to patients with higher baseline CD4 cell count. The proportion of the patients with baseline CD4 cell count < 50, 50-99, 100-149 and >150 cells/μl who failed to attain an increase of greater than 50 cells/μl despite suppressed viremia were 1.66%, 4.60%, 7.30%, and 11.9% respectively.

Table 9 Results of a ‡‡‡logistic regression model predicting the risk of discordant response at 12 months of ART.

Variable		Odd Ratio	P value	95% CI	
Age					
	14-24	Reference group			
	25-34	1.6	0.02[a]	0.5	5.4
	35-44	2.4		0.7	8.2
	≥44	4.3		1.2	15.4
Sex					
	Females	Reference group			
	Males	1.6	0.1	1.0	2.6
Clinical stage					
	Stages 1, 2 & 3[b]	Reference group			
	Stage 4	1.3	0.3	0.9	0.4
Baseline CD4 cell count (cells/μl)					
	0-49	Reference group			
	50-99	2.7		1.1	6.3
	100-149	4.0	P<0.01[a]	1.7	9.2
	≥150	6.8		3.0	15.7
Baseline viral load per one log increase		0.9	0.4	0.6	1.2
Prior TB[c]					
	No	Reference group			
	Yes	0.9	0.5	0.5	1.4

[a] Wald test computation which gave a combined P-value for the following variables: Age groups and Baseline CD4 cell count. The test prevents Type 1 error which can occur when comparing across catergories within groups.

[b] The non AIDS clinical stage (reference group) comprise stages 1, 2 & 3. Stage 4 was the AIDS clinical stage.

[c] The prior TB variable referred to the history of TB infection before inception of ART.

‡‡‡ The proportion of patients with discordant immune response in this cohort was 4.16% (see Table 8) after one year of ART. The computation had 1851 patients.

The patients with baseline CD4 cell count greater than 150 cells/μl had the greatest risk (Table 9) of discordant response (OR, 5.34, 95%CI 1.93; 14.7) compared to other lower baseline categories.

4.4.3 The subsequent survival among patients with immune discordant response and non-discordant CD4 response

The clinical progression to death between discordant and non discordant responses beyond 12 months differed, though not dramatically, in crude analysis (p=0.11[§§§]). For example, at 2 years, 2.1% of the patients with non-discordant response had died compared to 4.5% of the patients with discordant responses (and this proportion remained constant throughout the follow up period) had died (Fig 4).

However, the multivariate Cox regression model adjusted for baseline characteristics and CD4 cell count reported that subsequent survival was only associated with CD4 cell count and baseline viral load beyond 12 months (P<0.01) (Table 10). Discordant status at 12 months was not independently associated with survival after adjusting for absolute CD4 count.

[§§§] Log-rank test for equality of failure functions

Figure 5 Kaplan-Meier curve showing the proportion of patients who died at a time beyond 12 months among patients with discordant CD4 response compared with non discordant CD4 response

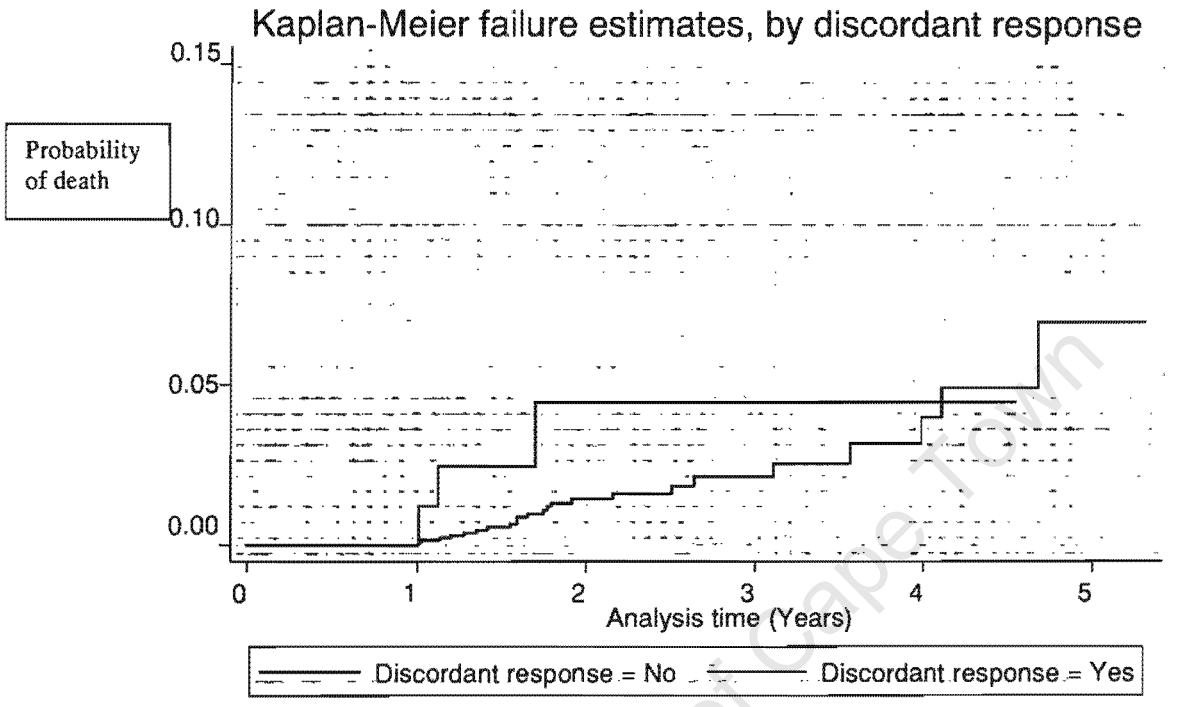


Table 10 Results of a Cox Proportional Hazard model to estimate the risk of death beyond one year in all patients

Variable		Relative Risk	P value	95% CI	
Age					
	14-24	Reference group			
	25-34	0.76	0.62[a]	0.16	3.65
	35-44	1.19		0.25	5.69
	≥44	1.46		0.26	8.29
Sex					
	Females	Reference group			
	Males	1.49	0.35	0.64	3.43
Clinical stage					
	Stages 1, 2 & 3[b]	Reference group			
	Stage 4	0.75	0.48	0.34	1.67
Baseline CD4 cell count (cells/µl)					
	0-49	Reference group			
	50-99	0.92		0.36	2.36
	100-149	0.30	0.73	0.06	1.48
	≥150	1.43		0.34	5.97
CD4 Cell count (cells/µl)		0.99	P<0.01	0.985	0.997
Discordant response					
	No	Reference group			
	Yes	0.83	0.79	0.21	3.29
Baseline viral load per one log increase		1.43	0.01	1.08	1.94
Prior TB[c]					
	No	Reference group			
	Yes	0.77	0.51	0.35	1.70

[a] Wald test computation which gave a combined P-value for the following variables: Age group and Baseline CD4 cell count. The test prevents Type 1 error which can occur when comparing across catergories within groups.

[b] The non AIDS clinical stage comprise stages 1, 2 & 3. Stage 4 was the AIDS clinical stage.

[c]The prior TB variable referred to the history of TB infection before inception of ART.

CHAPTER 5 - DISCUSSION AND CONCLUSION

This was the first analysis of patterns of and associations with CD4 response in patients accessing triple-drug antiretroviral therapy (ART) in Khayelitsha township in Cape Town, South Africa. The analysis included 3373 ART naïve HIV-infected patients followed up for up to four years in three ART clinics in Khayelitsha. These clinics have been providing ART to HIV-infected people since 2001.

Patterns of CD4 response

Males in this cohort had a lower median baseline CD4 cell count compared to females. The variability of CD4 cell count across the cohort increased from baseline to 48 months on ART (Fig 3). In the cohort there was a biphasic pattern of CD4 recovery on ART: median gains in CD4 cell count were significantly higher in the first six months compared to the subsequent months. Thus there was an initial rapid recovery of CD4 cells in the first 6 months followed by a more gradual recovery. Importantly there was continued gain in CD4 count throughout the 48 month period, suggesting that patients may potentially improve to CD4 count values in the normal range if they continue ART with suppressed viral loads for sufficient time.

These findings accord with what is known about immunological recovery on ART. The phases of CD4 cell count recovery during ART have previously been described to be divided into two parts. HIV disease is associated with immune dys-regulation and immune cell activation which promotes sequestration of $CD4^{+}CD45Ro^{+}$ memory T cells in lymphoid tissue (Autran et al. 1997). The suppression of viral load during ART in the first three to six months triggers the rapid redistribution of memory T-cells trapped in the lymphoid tissue and reductions in apoptotic cell death during this time period (Carcelain

et al. 2001 and Lawn et al. 2006). The second phase is more gradual and occurs beyond six months. This phase is associated with naïve-T-cell regeneration in the thymus (Autran et al. 1997).

The pattern of CD4 cell count gain is also explained by work done by Pido-lopez et al. (2003). In the early period of HIV infection the functional capacity of the thymus gland is greatly reduced. This limits its capacity to produce new cells in the early phase of ART (Pido-lopez et al. 2003). However as the patient continues on ART, the viral burden decreases and the thymus recovers some of its function to aid immune restoration (Pido-lopez et al. 2003). The increase in thymic function was reported by Douek et al. (1998) to be associated with increases in CD4 T cell numbers during ART. This recovery is slow due to the slow repopulation of naïve CD4 T-cells by the thymus gland (Hunt et al. 2003).

Although our findings showed that CD4 cell counts continued to increase slowly over the four year follow up period, the question that remains is whether these increases can be sustained beyond this. Moore and Keruly (2007) reported that patients with good virological response below 1000 or 400 copies/ml showed a plateau effect on CD4 response. Patients with lower CD4 cell counts leveled off below normal ranges. However, a study by Mocroft et al. (2007) has recently shown that in patients with viral load suppression below 50 copies per ml, those with baseline CD4 cell count above 350 cells/μl can attain normal CD4 cell count in 5 years, with ongoing increases also in patients with lower CD4 cell count.

Commenting on these findings, Maartens & Boulle (2007) said that patients maintaining maximum virological suppression on ART would eventually achieve normal CD4 cell counts including those with lower baseline CD4 cell count.

Associations with CD4 response

CD4 gains were more pronounced in patients with viral load suppression throughout the follow up time, followed by gains in patients with suppressed viral load at two successive time points.

At six months, lower gains in CD4 cell count were associated with older age, male gender, and higher baseline CD4 cell count. Higher gains in CD4 cell count at six months were also associated with higher baseline viral loads and a suppressed viral load at six months. The variables associated with lower CD4 cell gains from 6 to 48 months were older age, male gender, lower baseline CD4 cell count, higher preceding CD4 cell count and not having a continuously suppressed viral load. A higher baseline viral load was also associated with greater CD4 cell count increase beyond six months of therapy.

Although rates of increase in CD4 cell counts appeared to decline with subsequent years of ART, the effect of the baseline CD4 cell count on the rates of CD4 cell change over time persisted. Patients with lower baseline CD4 cell count (<50 cells/μl) had the greatest rates of CD4 cell count increases compared to those with higher baseline CD4 cell count after 6, 12 and 24 months of ART. There were greater rates of CD4 cell counts increase in the lower baseline categories but this phenomenon decreased over time.

Lawn et al. (2006) had similar findings in which they suggested that patients with baseline CD4 cell count less than 50 cells/μl were least likely to be immunological non-responders to ART. Despite higher rates of CD4 cell count rise, however, these patients had the greatest risk of failure to attain absolute CD4 cell count greater than 200 cells/μl due to their starting from lower baseline CD4 cell counts (Lawn et al. 2006).

Explaining the implication of the relationship between the length of time to attain 200 cells/μl gain in CD4 cell count and the rates of increase, Lawn et al. (2006) noted that prolonged periods below the threshold "safe" CD4 cell count of 200 cells/μl rather than diminished rates of CD4 cell count increase was likely to be a strong marker of high morbidity and mortality during the earlier months of ART. Equally important, CD4 cell count at baseline is the strongest prognostic factor for AIDS and death (Battegay et al. 2006). These authors highlighted that patients who start ART with fewer than 200 cells/μl were at the highest risk of clinical progression.

The patterns of immune recovery in our study are comparable to a study by Mocroft et al. (2007). These authors studied normalization of CD4 cell count in 1517 antiretroviral naïve HIV positive patients with viral suppression below 50 copies/ml. Their study was different from ours because we defined viral suppression as a viral load below 400 copies/ml. But similar to our study, they found that the rate of CD4 cell count increase diminished with increasing time from start of cART (combination antiretroviral therapy) in patients who start ART with lower baseline CD4 cell count. They also established that although patients with lower baseline CD4 cell count had significant increases, over time,

these patients had consistently lower current absolute CD4 cell counts compared to patients who would had started with a higher baseline CD4 cell count.

Viral load at baseline and at six months and beyond stood out to be the main determinants of CD4 cell count gains. The gains in CD4 cell count explained by baseline viral load were lower than the gains attained when viral load was suppressed (compared to those not suppressed) at any given time point. In this regard, Lawn et al. (2006) suggested that there is a positive correlation between CD4 cell sequestration in the lymphoid tissue and plasma viral load.

The presence of replicating HIV in the body stimulates cell sequestration. ART in the first 3-6 months suppresses viral load replication, promoting the redistribution of cells from the lymphoid tissue into the blood. The degree of initial CD4 rise due to this redistribution is likely to be related to the degree of sequestration prior to ART, which is in turn related to the starting viral load. After six months, however, the baseline viral load becomes less important and instead, the continuously suppressed viral load at any time point becomes a major determinant of CD4 response. This is likely to be in a large part due to suppressed viral load being a marker of adequate adherence to ART.

Sequestration of memory T-cells in HIV-infected untreated patients and subsequent redistribution on ART may explain why patients with advanced disease have more gains in CD4 cell count. These patients have higher viral loads and thus greater immune activation and sequestration of memory cells. The capacity for redistribution when the viral load is suppressed on ART is therefore likely to be greater.

As demonstrated by the findings of our study, less gain in CD4 cell count was strongly associated with older age both at six months and beyond six months.

From findings of their study, Giordano et al. (2003) reported that women repopulate their peripheral CD4 cells in response to virus suppression more quickly than men do. Our study confirms the results of their study. The authors suggested that women may have increased peripheral redistribution of memory CD4 cells from lymphoid tissue in response to viral suppression as a result of the effect of ART. To explain higher CD4 count gains in females beyond six months of ART, the authors also suggested that women may have more thymic output of naïve CD4 cells in response to ART compared to men. Another study was done by Finkel et al. (2003) to analyze the immunological response difference between women and men in patients who respond virologically. The authors reported that larger increases in CD4 T-cells were observed in women than men. Similar to the explanation given by Giordano et al. (2003), Finkel et al. (2003) cited significant immunological differences which exist between males and females.

Also explaining the differences in CD4 response, Giordano et al. (2003) cited pharmacokinetic differences between males and females. They suggested that these differences may result in women accumulating higher antiretroviral drug levels, which subsequently lead to greater viral suppression and greater increases in CD4 cell count. These authors suggested that women are more likely to achieve viral load suppression below detection limits, hence they have a superior immune response compared to men.

Discordant responses

At 12 months the proportion of patients with discordant response (having immunological non-response despite a fully suppressed viral load) was 4.2%. This percentage is lower than that reported from high income nations which stood between 20-30% (Tuboi et al. 2007). Lawn et al. (2006) also reported similar findings in which the proportion of discordancy was 7%. Their study was conducted in a similar setting to the researcher `s study, in another ART clinic in Guguletu, Cape Town, South Africa. The authors attributed reasons for the differences to high income countries. These reasons likely apply to our study too. The reasons included the fact that their study included only antiretroviral naïve patients and there were also low rates of primary drug disease resistance and high treatment adherence rates.

The risk of discordant response increased with increasing baseline CD4 cell count and increasing age. Our study obtained similar findings to a study done by Tuboi et al. (2007). This study included 3111 ART-naïve patients and it assessed the frequency and risk factors for discordant responses in resource limited settings. Some of their findings included the association of discordant response with older age and baseline CD4 cell count. The authors noted that these findings were also similar to the findings of similar studies done in high income nations. Unlike our study, their analysis defined discordance at six months whereas our study defined discordance at 12 months.

In explaining reasons why the risk of discordant response increases with increasing baseline CD4 cell count, Tuboi et al. (2007) attributed it to the non linear nature of CD4 cell count increase after ART initiation across different baseline CD4 cell count

categories. This was exemplified by the fact that in our study, the increases in CD4 cell count in lower baseline categories in the first six months was greater than higher baseline categories.

The finding of our study that the risk of discordant response increases with age was consistent with the hypothesis that immune restoration is dependent on the thymus activity which becomes dysfunctional with increasing age (Teixeira et al. 2001). Increasing age increases the likelihood that the regenerative capacity of the thymus to produce naïve T-cells in response to viral load suppression will fail. Thymic involution during ageing thus has a negative impact on ART-induced CD4 T-cell reconstitution (Graber et al. 2000).

Clinical outcomes

The CD4 cell count at 12 months was the only variable associated with subsequent survival after one year of ART. We found no difference in mortality after 12 months between those who had discordant response at 12 months and those that did not. Similar to our study, Wood et al. (2000) reported absence of differences in progression to AIDS or death between discordant CD4 cell responders (both immunologic and virologic) and concordant virologic/immunologic responders.

Our study mirrors findings of several studies which reported that CD4 cell count as a important predictor of survival as compared to viral load. For example, a study by Piketty et al. (2001) which involved 150 HIV infected patients with discrepant virologic and immunologic responses in the first 12 months of ART were followed up for 30 months to

assess their clinical outcome. These authors concluded that the CD4 cell was a marker over plasma HIV load for predicting clinical outcome in patients who do not achieve full immunologic and virologic responses. Although we analyzed patients with virologic response but no immune response, the findings of Piketty et al. (2001) are relevant to our study. Similar to our findings, CD4 cell count stood out to be the only predictor of death amongst other variables such as viral load, age and clinical stage.

The strengths and limitations

The first limitation of this study arose from using the population average models which could not allow us to specify population distribution. Hence we could not report estimates of measures of variability between and within subjects. The random effects models could not be used because we modeled CD4 cell count changes at six months intervals, not the absolute measurements. The strength in our analysis rested in the incorporation of the autoregressive covariance structure in our computation. This was justified by increased variability of CD4 cell count over the 48 months follow up. Although our study was strengthened in this regard, some patients were dropped during the computation of the correlation because they had fewer than two CD4 cell count measurements.

The other limitation of the study was the fact that in the clinics' database suppressed viral load was captured as a viral load below 400 copies/ml and not 50 copies/ml. This was a drawback because we could not directly compare our study to those studies which used patients with under 50 copies/ml. These studies could possibly show different CD4 count change profiles to our study because of this difference in definition of viral load suppression.

The history of TB infection did not give us much information. TB infection during ART by the time of the analysis was not captured. HIV positive patients on TB treatment when they start ART had higher baseline CD4 count than those patients that are infected but not on TB treatment. We could not control for the effect of TB treatment in the analysis.

The findings of this study are strengthened by the fact that it was conducted in a homogenous population. The patients were all ART-naïve and received standard triple drug regimen with uniform follow-up time points. Despite the fact that the analysis included three different sites, these sites were all located in Khayelitsha and followed standardized clinical protocols. The findings of this study are likely to be generalisable to other ART programmes in sub-Saharan Africa.

Implications

What are the implications of the findings of these results for the Khayelitsha clinics and other settings in sub-Saharan Africa? Clinicians need to be aware of the immune reconstitution inflammatory syndrome**** (IRIS) during the early days of ART. The development of IRIS is associated with low CD4 cell count especially in patients below 50-100 cells per μl and usually occurs between 2-8 weeks after the inception of ART (Battegay et al. 2006). This is the same range which most of our patients start their therapy. Furthermore, the same low baseline category is also associated with higher gains in CD4 cell count. It is therefore important not to underplay the potential development of IRIS during the early weeks of increases of CD4 cell count in patients taking ART.

**** According to (Battegay et al. 2006 :(6) 284), IRIS is "thought to result from inflammatory reactions to previously asymptomatic pathogens".

The frequency of discordant response was low in our data. It is in fact less common in low income countries as compared to high income countries (Lawn et al. 2006). Discordant response in the first year did not have an impact beyond 12 months on both CD4 cell count change and subsequent outcome (death) in the Khayelitsha HIV programme.

Immune recovery is possible in patients with the lowest baseline CD4 counts despite their increased risk of death and morbidity. It is possible that patients who start ART with advanced disease will eventually attain normal CD4 cell counts, so long as virological suppression is maintained. This demonstrates the need for monitoring of viral load levels if feasible. Gender, age and baseline markers of disease progression (CD4 count, WHO stage and viral load) are strong determinants of CD4 cell response on ART in patients in the Khayelitsha clinic.

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