EVOLUTION OF THE CD4 COUNT IN THE FIRST 12 MONTHS FOLLOWING INITIATION OF ANTIRETROVIRAL THERAPY IN A SOUTH AFRICAN PUBLIC-SECTOR PATIENT POPULATION

MFUNDO FALETHU MATHENJWA

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MFUNDO FALETHU MATHENJWA

Thesis submitted in part-fulfilment of the requirements for the degree of Master of Medicine (Medicine) in the School of Clinical Medicine, University of KwaZulu-Natal

Supervisor

Prof Richard J Hift

Prof Nombulelo Magula

School of Clinical Medicine, University of KwaZulu-Natal, South Africa

DECLARATION

I, Mfundo F Mathenjwa, declare that:

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Dr MF Mathenjwa

Candidate

Prof RJ Hift

Supervisor

11 January 2017

DEDICATION

This thesis is dedicated to my parents: Simeon and Linah Mathenjwa.

ACKNOWLEDGMENTS

I express my appreciation to my supervisors, Professors Richard Hift and Nombulelo Magula.

TABLE OF CONTENTS

ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
CCR5	C-C Chemokine receptor 5
HIV	Human immunodeficiency virus
IRIS	Immune reconstitution inflammatory syndrome
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleic reverse transcriptase inhibitor
TB	Tuberculosis
UTT	Universal test and treat
WHO	World Health Organisation

PAGINATION AND REFERENCING

The pages of the dissertation are numbered consecutively at the bottom of each page. Chapter 2, which is presented in manuscript form is additionally numbered separately in the top right corner.

The introductory chapter, Chapter 1, is referenced with bold numbers in square brackets thus: **[1].** The bibliography is found at the end of that chapter. Chapter 2, which is presented in manuscript form, is separately referenced with superscript numerals thus: [1]. The approved protocol (Appendix 1) is also separately referenced, and the bibliography will be found at the end of each of these sections.

CHAPTER 1
INTRODUCTION

INTRODUCTION

LITERATURE REVIEW

CD4 count and HIV infection

The CD4+ T lymphocyte is the primary target of HIV infection **[1]**. The CD4 count reflects the extent of the resultant immune suppression. It is calculated from the CD4 cell percentage and total white blood cell count. The CD4 count reference values vary considerably amongst different laboratories **[2]**. For most laboratories, the mean CD4 count is of the order of 500-1300 cells/mm^3. Untreated HIV-infected individuals, experience a yearly CD4 count decline of 50-80 cells/mm^3. The pattern of decline is usually slow, taking ten years for a newly infected person to progress to the acquired immunodeficiency syndrome (AIDS). There is however great variation among individuals **[3-6]**. For some patients, known as rapid progressors, disease progression occurs within a couple of years. A major criterion for this is the demonstration of more than two CD4 T cell measurements below 350 cells/mm^3 within 3 years after seroconversion, with no value $\geq$ 350/mm^3. By contrast, long-term non-progressors show little or no decline in CD4 count over an extended period **[5]**.

Among those with HIV infection, the CD4 count is therefore the major indicator of immunodeficiency. It was for many years the principal factor in deciding whether to initiate antiretroviral therapy (ART) **[7,8]**. More recently there been a major shift in this approach, with initiation of ART being recommended for all HIV-positive individuals, irrespective of CD4 count **[9,10]**. The CD4 count is an essential parameter in monitoring both the progress of the disease and the response to ART. Initiation of prophylaxis against opportunistic infections is typically dependent on demonstration of a low CD4 count **[7]**. Conversely, viral suppression typically leads to quantitative and qualitative immunological reconstitution**[11-13]**.

A sustained CD4 count greater than 200 cells/mm^3 dramatically reduces the risk of opportunistic infections such as cytomegalovirus, *Pneumocystis jiroveci*, *Mycobacterium avium* complex, *Toxoplasma gondii*, and *Cryptococcus neoformans*, allowing primary and secondary prophylaxis for many opportunistic infections to be discontinued **[14,15]**. For this reason, a CD4 count of less than 200 cells/mm^3 has traditionally been used as an indication for prophylaxis against *Pneumocystis jiroveci* pneumonia. International best practice however now

suggests a threshold of 350 cells/mm^3 **[16]**. The risk of tuberculosis (TB), though markedly reduced by ART, remains higher than that in the general population **[17-19]**. It is recommended that all patients receiving ART in South Africa should be considered for isoniazid prophylaxis **[15]**, since prophylaxis has been shown to result in a 37% reduction in incident TB **[20]**. Patients who are diagnosed with cryptococcal meningitis should remain on prophylactic fluconazole for a minimum of 1 year following acute treatment. This may be discontinued in patients on ART who demonstrate viral suppression and a CD4 count>100 cells/mm^3 for at least 3 months **[21]**. However, even with control of the conventional opportunistic infections, there is still a challenge in attaining sufficient immunocompetence to prevent non-conventional opportunistic complications such as HIV-associated cardiomyopathy, steatohepatitis and HIV associated nephropathy **[22,23]**.

Viral load and viral suppression

The HIV-1 viral load is an indication of the number of copies of HIV-1 RNA per millilitre of plasma. Despite HIV being an intracellular pathogen, the HIV-1 viral load in plasma is a precise barometer of the extent of infection and the stage of viral replication [24]. It is essential in gauging virological response to pharmacotherapy. The HIV-1 viral load measurement indicates the number of copies of HIV-1 RNA per millilitre of plasma. Although HIV ultimately resides within cells, the plasma measurement is an accurate reflection of the burden of infection and the magnitude of viral replication It is critical in monitoring virological response to therapy. The primary aim of combination antiretroviral therapy is maximal and durable suppression of viral replication **[7]**. This delays development of drug resistant mutations, preserves and increases CD4 cells and ultimately results in better clinical outcomes. A failure to achieve viral suppression is an indication for a switch to a second or third line ART regimen provided that compliance has been addressed **[7]**.

Assessing response to antiretroviral therapy

Failure of suppression of viral load is a critical step in the early recognition of treatment failure since a rise in CD4 count and a deterioration in clinical response typically take much longer to develop **[7]**. With an effective ART regimen, viral suppression to undetectable levels should be achieved within 3 to 6 months after initiation of therapy **[2,25]**. Frequent monitoring of CD4 counts, especially in those with higher counts, above 300 cells/mm^3, is generally not required

in patients with consistently suppressed viral loads. South African practice has been to measure the CD4 count annually in patients receiving ART whose condition is otherwise satisfactory **[26]**. The most recent guidelines however recommend that annual CD4 count measurements should be regarded as optional in patients on ART with a CD4 count >500 cells/mm^3 **[7]**.

The detection of treatment failure is an important part of the management of patients receiving ART. Virological failure is variously defined as the inability to achieve or maintain suppression of viral replication to an HIV RNA level less than 200 copies/ml **[7]**, or as a viral load above 1000 copies/ml based on two consecutive viral load measurements three months apart in a patient who has been on ART for at least six months and has had adherence support **[27]**. Immunological failure is indicated by a CD4 count ≤ 250 cells/mm^3 following clinical failure (see below), or a persistent CD4 count below 100 cells/mm^3. Clinical failure is defined by the development of new or recurrent clinical conditions indicative of severe immunodeficiency after 6 months of effective treatment **[27]**.

Immune reconstitution inflammatory syndrome

The immune reconstitution inflammatory syndrome (IRIS) is characterised by strengthening of a previously weak immune system resulting in paradoxical clinical deterioration due to a recovery in the immune system, allowing it to mount a strong inflammatory response to a pathogen. It is usually self-limiting and does not require discontinuation of ART, though it may occasionally be life-threatening **[28,29]**. In patients not on ART presenting with cryptococcal meningitis or TB meningitis, initiation of ART is typically deferred for a short period to lessen the risk of IRIS **[30]**. National guidelines in South Africa recommend a 2-week interval between initiating antituberculous therapy and initiating ART **[31]**.

Antiretroviral therapy in South Africa

South Africa has the highest HIV prevalence in the world, and additionally the largest ART program **[32,33]**. The national ART programme in South Africa was launched in April 2004. Initially antiretroviral therapy was made available to all public-sector adult patients with a CD4 count of less than 200 cells/mm^3 as well as those with Stage 4 clinical disease. In 2010 the program was expanded to include pregnant and TB-co-infected subjects with a CD4 count of less than 350 cells/mm^3, and in August 2011, to enrol all patients with a CD4 count of less than 350 cells/mm^3 in line with the then World Health Organisation HIV Guidelines. As of April

2013, all patients co-infected with drug-sensitive or resistant TB were initiated on ART irrespective of CD4 count. This coincided with the introduction of fixed drug combination ART for patients who are initiated on ART for the first time **[7,8]**. This helps reduce the pill burden, thus facilitating compliance. A protocol for the phased introduction of fixed drug combination ART to patients with other co-morbidities, such as TB and cryptococcal meningitis was developed, resulting in classification of patients according to different eligibility groups. HIV positive women who are pregnant or breastfeeding, patients with a low CD4 of less than 200 cells/mm^3 patients with stage 4 illness irrespective of CD4 count and patients co-infected with TB with CD4 count <50 cells/mm^3 were all now be eligible for initiation of ART within seven days, bringing the South African guidelines into convergence with the WHO 2013 consolidated guidelines on use of antiretroviral drugs **[34]**

With effect from 1 September 2016, in terms of a *Universal Test and Treat* (UTT) policy, all HIV-positive individuals presenting to South African public health institutions are eligible for immediate initiation of ART, with certain vulnerable groups expressly prioritised for rapid enrolment **[35]**. The recent national UTT policy brings South African practice into conformity with recent World Health Organisation guidelines **[10]** and the 2016 update to the 2014 USAID guidelines **[7]**, both of which recommend that ART should be initiated among all adults with HIV regardless of CD4 count. South Africa is committed to the 90-90-90 targets set by the Joint United Nations Programme on HIV/AIDS(UNAIDS) **[36]**, which state that by 2020, 90% of all people living with HIV will know their HIV status, 90% of all people living with diagnosed HIV infection should receive sustained ART, and 90% of all people receiving ART will have viral suppression.

Current South African practice in initiating adult patients on antiretroviral therapy

South African patients in whom ART is initiated are currently managed in accordance with the South African National consolidated guidelines of 2015 **[37]**, as modified by the September 2016 policy document mandating universal test and treat **[35]**. Patients are advised on how to avoid HIV transmission to sexual partners and children. They are initiated on isoniazid prophylaxis if asymptomatic for TB. They are also provided with counselling on nutrition and contraception and to do annual Papanicolaou smear.

Patients are screened for syphilis and active TB at the time of first diagnosis. Baseline CD4

cell count and biochemical profile including full blood count, renal and liver function tests are performed. Follow up is subsequently every 12 months for clinical and biochemical review. This entails screening for opportunistic infections and drug adverse effects. Of paramount importance is the 12-monthly monitoring of immunologic and virologic response, which involves monitoring of the CD4 count and viral load.

A typical first-line South African antiretroviral therapy regimen includes two nucleoside reverse transcriptase inhibitors (NRTIs), currently tenofovir, lamivudine or emtricitabine, and one non-nucleoside reverse transcriptase inhibitor (NNRTI), currently efavirenz **[37]**. Stavudine, initially widely used as first-line therapy, has been phased out with all patients currently on a stavudine containing regimen being moved to a tenofovir-containing regimen, provided there are no contraindications. Stavudine is to be used only under specific circumstances, for example in patients with renal failure and severe anaemia, in which case the use of tenofovir and zidovudine are respectively contra-indicated, Abacavir may also be used under the above circumstances if stavudine is contraindicated **[37]**. This agent is however costly and carries a small risk of hypersensitivity reactions. The use of nevirapine has been superseded by the use of efavirenz given the more favourable toxicity profile of the latter.

Failure of this regimen may require switching to a protease inhibitor (PI)-containing second line regimen (ritonavir/lopinavir combination). This is done with expert advice. Failing any second line regimen, patients are referred for specialist opinion for a regimen based on genotype resistance testing **[35]**. These patients receive supervised care and are managed centrally by the National Department of Health. A third line regimen comprises darunavir/ritonavir, emtricitabine or lamivudine, tenofovir or zidovudine with or without raltegravir with or without etravirine, based on genotype scoring **[7]**.

THE CURRENT STUDY

Background to the current study

South Africa has adopted the UTT strategy as of 1 September 2016 **[35]**. This might seem to make the use of CD4 count as a tool in management somewhat redundant as patients are initiated on ART regardless of CD4 count. However, the continued importance of CD4 testing at both initiation and subsequently has been described in the preceding sections; principally in

terms of the decision to initiate and withdraw prophylaxis for opportunistic infections **[21]** and the accumulating evidence that a low initial CD4 count and immunological failure during therapy are associated with worse outcomes in the long-term, even in the presence of adequate viral suppression **[38]**.

The problems inherent in administering a public-sector ART programme of the magnitude of the South African programme are acknowledged **[33]**, and there is expected value in studying the trend in CD4 count in response to ART administered in a South African public-sector setting. In this study, the candidate set out to examine the extent of the increase in CD4 count in the first year after initiation of ART in a local setting, and to examine some of the factors which may affect it.

Research question

- What is the observed increase in CD4 count after 12 months' ART therapy in patients attending a large South African public hospital?

Hypothesis

- Properly prepared patients on South African public hospital who demonstrate viral suppression will demonstrate significant improvement in CD4 count.

Aims

- To determine the magnitude of the increase in CD4 count in patients demonstrating viral suppression following 12 months ART, the association of this with clinical well-being and its association with other parameters which might remote or retard immune reconstitution.

Objectives

- To measure the increase in CD4 count in patients on ART
- To correlate the increase in CD4 count with changes in haemoglobin concentration and weight
- To document factors which might influence the magnitude of the CD4 response: sex, CD4 count at time of initiation of ART

- To document and describe the incidence of opportunistic infections in patients on ART with viral suppression
- To document the incidence of IRIS in patients on ART

Methodology

The candidate conducted a retrospective chart review of patients enrolled at the Philani Clinic (the HIV clinic attached to King Edward VIII Hospital, Durban, South Africa). A power calculation indicated that 230 subjects were necessary to demonstrate a difference in CD4 count between baseline and one year, significant at the 5% level with a beta error of 20%. A pro-forma data capture sheet was designed and used to facilitate the collection of all data relevant to the aims and objectives of the study.

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CHAPTER 2

Evolution of the CD4 count in the first 12 months following initiation of antiretroviral therapy in a South African public-sector patient population

TITLE

Evolution of the CD4 count in the first 12 months following initiation of antiretroviral therapy in a South African public-sector patient population.

AUTHORS

Mathenjwa M
Hift R
Magula N

AFFILIATIONS

Department of Medicine, University of KwaZulu-Natal, Durban, South Africa

CORRESPONDING AUTHOR

Prof RJ Hift
School of Clinical Medicine
Private Bag X7
Congella
South Africa
4013

WORD COUNT

Abstract	416
Text	2946
Tables	5
Figures	1

ABBREVIATIONS

ART	Antiretroviral therapy
CCR5	C-C Chemokine Receptor type 5
HIV	Human immunodeficiency virus
IQR	Interquartile range
IRIS	Immune reconstitution inflammatory syndrome
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
SD	Standard deviation
TB	Tuberculosis
WHO	World Health Organisation

ABSTRACT

Aim

We studied the 12-month clinical and CD4 count response in patients newly initiated on antiretroviral therapy in a South African public hospital.

Background

The CD4 count and viral load are closely linked to human immunodeficiency virus (HIV)-related illness and mortality. Numerous studies have shown that initiation of ART can improve quality of life and longevity in HIV-1 infected patients.

Methods

We performed a retrospective chart review of 257 patients initiated on ART over one year in whom viral load suppression was documented. We monitored the change in CD4 count, viral load, weight and haemoglobin concentration over the first 12 months, and documented the incidence of opportunistic infections and of the immune reconstitution inflammatory syndrome (IRIS).

Results

Of 257 patients, 219 had complete viral suppression; 30 showed incomplete suppression with a final load <400 copies/ml and 8 subjects had a final viral load <1000 copies/ml. The mean CD4 count at baseline was 139 [IQR 63-195] cells/mm^3. The CD4 count at baseline was significantly lower in males (90 [IQR 24-180] cells/mm^3) than females (148 [IQR 88-199] cells/mm^3). Opportunistic infections were observed in 87 of 257 patients (33.9%) and were significantly more frequent in males (52%) than females (27%). Sixty-eight patients had tuberculosis (TB); 17 had cryptococcal meningitis (17) and 13 had oesophageal candidiasis. Opportunistic infections were significantly more common in males and in patients with lower CD4 counts at entry. Seven patients developed IRIS. All had TB; two presented additionally with cryptococcal meningitis. Comparison of values at 12 months with baseline showed a significant increase in CD4 count from 139 to 327 cells/mm^3 (p<0.0001), weight (67.3 kg-71.4 kg) (p<0.0001) and haemoglobin (11.1-11.9 g/dL) (p<0.0001). The increase in CD4 count was

similar across the full range of baseline counts, including those with an initial count less than 50 cells/mm^3. The probability of a satisfactory response in CD4 count was not affected by the presence of an opportunistic infection. Patients treated with nevirapine showed a significantly higher eventual CD4 count, even after controlling for the baseline CD4 count, than patients treated with efavirenz (p=0.015).

Conclusion

Males present to our service with a lower baseline CD4 count and higher rate of opportunistic infections than females. In patients in whom complete or partial viral suppression was obtained, initiation of ART resulted in a significant increase in CD4 count in patients irrespective of age, sex, baseline CD4 counts and the presence of an opportunistic infection. We conclude that antiretroviral therapy is highly effective in our population and comparable in efficacy to international experience.

INTRODUCTION

The CD4+ T lymphocyte is the primary target of HIV infection[1], and the CD4 count reflects the extent of the resultant immune suppression. The primary aim of combination antiretroviral therapy (ART) is maximal and sustained suppression of viral replication[2]. Most patients who achieve and maintain an undetectable HIV plasma RNA level on ART will demonstrate a substantial increase in their peripheral CD4 count[2-4]. A sustained CD4 count greater than 200 cells/mm^3 dramatically reduces the risk of opportunistic infections, and a treatment-associated rise in CD4 count will permit the discontinuation of prophylaxis for opportunistic infections such as *Pneumocystis jiroveci*, *Toxoplasma gondii*, and *Cryptococcus neoformans*[5,6].

Immune reconstitution in response to ART occurs most quickly in the first two years after commencing ART, and the CD4 count rises more slowly thereafter[7,8]. An average increase of 93-151 cells/mm^3 over baseline values has been reported for the first six months of therapy with a subsequent average increase of 22-36 cells/mm^3 per year for the first four years of therapy thereafter[9-11]. ART-induced increases in CD4 count are more variable from patient to patient than is the reduction in viral load, and may be affected by factors such as the elapsed time between HIV seroconversion and initiation of ART, pre-ART CD4 count, the degree of viral suppression, the presence of clinical AIDS before initiation of treatment, hepatitis C coinfection and the Δ32 CCR5 genotype[3,10,12-14]. Though in general the type of regimen used is not thought to be a major predictor of the likelihood of a poor immunological response[15,16], it has been suggested that zidovudine-containing regimens and male sex may be associated with lower responses[17]. Concurrent opportunistic infections, commonly tuberculosis (TB), may blunt ART-induced CD4 count improvement. A history of HIV-associated TB prior to commencement of ART[18] or incident TB during ART[17] are also reportedly associated with lower CD4 count recovery. This has however not been the experience in South Africa where patients presenting with TB at the time of initiation of ART had similar increases in CD4 in comparison with other patients[19].

A minority of individuals do not achieve full immune reconstitution, even after prolonged viral suppression[8], particularly those in whom initiation of ART was delayed until after their CD4 cell count had dropped below 200 cells/mm^3 [2,20,21]. Studies of the recovering immune response show this to be a complex process[22], and the mechanisms underlying incomplete immunological recovery are not understood. Immune activation as measured by co-expression

of CD38 and HLA-DR on T-cells is a strong predictor of CD4 cell loss and disease progression in untreated HIV infection[23-25], and this may be a feature of retarded immunological recovery in response to ART as well[26,27]. Incomplete immunological recovery has clinical consequences, and failure to restore a normal CD4 count following ART is associated with increased morbidity and mortality[28].

The addition of CD4 count and viral load monitoring to clinical monitoring is associated with improved health and survival[2,29]. Frequent monitoring of CD4 count is in general not required in patients with consistently suppressed viral loads, particularly where the CD4 count is above 300 cells/mm^3. South African practice has been to measure the CD4 count annually in patients receiving ART whose condition is otherwise satisfactory[30]; the updated US guidelines continue to recommend this for otherwise stable patients receiving ART beyond two years with a CD4 count below 500, but indicate that it is optional for patients with a CD4 count above 500[2].

CD4 count thresholds were for many years the principal factor in deciding when to initiate ART[2,31]. Currently ART is recommended for all HIV-positive individuals, irrespective of CD4 count[32,33], and this has now been incorporated into South African public sector guidelines as part of the *universal test and treat* (UTT) policy[34]. While CD4 count is no longer a factor in initiating treatment, it retains its importance in predicting susceptibility to opportunistic infections and in predicting long-term outcome even in patients with a satisfactory virological response to ART[28,35].

The South African ART programme is the largest in the world and, given the country's status as a developing economy, successful program delivery is challenging[36]. We believe there is value in studying the trend in CD4 count in response to ART administered in a South African public-sector setting. In this study, we describe the extent of the increase in CD4 count in the first year after initiation of ART in a local setting, and examine some of the factors which may affect it.

SUBJECTS AND METHODS

We performed a retrospective chart review of patients enrolled at the Philani HIV Clinic at King Edward VIII Hospital, Durban, South Africa. We included patients 18 years and older, commenced for the first time on ART from January 2010, who had satisfactorily completed at

least 12 months of follow-up, including a subsequent viral load, CD4 count and clinical and laboratory data. We excluded patients with incomplete data, poor adherence to medication (which we defined as a failure to attend any scheduled visit to the hospital pharmacy for treatment collection), documented psychiatric illness or a final viral load greater than 1000 copies/ml. The study was adequately powered to demonstrate a difference in pre-treatment and post-treatment CD4 count at the 5% level with a beta error of 20%.

Age, sex, viral load, CD4 count, weight, haemoglobin concentration and regimen type were documented at initiation and on completion of 12 months' follow-up. We documented any preceding or intercurrent opportunistic infections, and any episode of the immune reconstitution inflammatory syndrome (IRIS). ART was commenced in patients with TB following at least 2 weeks of anti-tuberculous treatment.

Ethical considerations

All data were anonymised. Individual patient consent was not required for this retrospective review. The study was approved by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (Approval Number BE 063/12), and was performed with the permission of Hospital Management.

Data management and statistical analysis

All data were entered on an Excel spreadsheet (Microsoft Corporation, Redmond WA). The observed change in CD4 count was correlated with demographic data (age, sex and race), presence of intercurrent infection, WHO HIV clinical stage, baseline CD4, ART adherence as reflected by clinic attendance, and use of other drugs known to interact with ART. Normally distributed data are reported as *mean (standard deviation, SD)* and were tested for significance using Student's t-test. Non-parametric data are reported as the *median [interquartile range, IQR*] and were tested for significance using the Mann-Whitney U test and Kruskal Wallis ANOVA. Correlations were tested using the Spearman rho test. Statistical calculations were performed using MedCalc version 16.8 (MedCalc Software bvba, Ostend, Belgium). A *p* value of 0.05 was regarded as significant.

RESULTS

We reviewed the records of 257 patients who had received ART for at least one year and demonstrated viral load suppression to less than 1000 copies/ml. All patients were of African ethnicity and none had received prior ART.

Patients were treated according to standard protocols in force at the time of initiation of ART. All received lamivudine, with either stavudine or tenofovir as the nucleoside reverse transcriptase inhibitor (NRTI), and nevirapine or efavirenz as the non-nucleoside reverse transcriptase inhibitor (NNRTI). Baseline parameters are shown in Table 1. There was no significant difference in age between males and females. Median baseline CD4 count was 139 [IQR 63-195] cells/mm^3 and was significantly lower in males than females ($p< 0.003$).

Eighty-seven of the 257 patients (33.9 %) had experienced opportunistic infections prior to initiation of treatment (Table 2). The three most prevalent opportunistic infections were TB (68), of which 24 were disseminated or extrapulmonary TB, cryptococcal meningitis (17) and oesophageal candidiasis (13). Patients who had previously experienced an opportunistic infection had a significantly lower CD4 count at entry (64 [IQR 24-168] cells/mm^3) then those who had not (161 [IQR 112-202] cells/mm^3, p=0.0001) and opportunistic infections were significantly more frequent in males (52 %) than females (27%) (p=0.001).

Viral suppression in response to ART was a requirement for inclusion in our study. 219 subjects showed complete viral suppression; a further 30 showed incomplete suppression with a final viral load <400 copies/ml, and in a further 8 subjects, <1000 copies/ml. Males and females did not differ in their probability of an incomplete response (p=0. 84).

We demonstrated a highly significant increase in CD4 count, weight and haemoglobin over the first 12 months of therapy (Table 3). Age did not correlate with the baseline CD4 count (p=0.74) or with the increase in CD4 count on therapy (r=-0.114, p=0.07). The probability of a satisfactory response in CD4 count was not affected by the presence of an opportunistic infection (p=0.14).

There was a highly significant correlation between the CD4 count at baseline and that reached at 12 months (Spearman's rho =0.643, p<0.0001). Though there was no correlation between the absolute increase in CD4 count and baseline CD4 level (p=0.17), with a mean increase of approximately 200 cells/ml in all patients with an initial CD4 count below 350 (Table 5), when

the increase is expressed as a percentage over baseline. This equates to a proportionately greater increase in patients with initially low values (Spearman's rho=-0.74, $p<0.0001$). A significantly larger increase in weight was observed in patients with lower CD4 counts at baseline (Spearman rho=-0.17, $p=0.006$). There was no significant correlation between initial CD4 count and change in haemoglobin ($p=0.43$).

Seven of the 257 study patients (2.7%) developed the immune response inflammatory syndrome (IRIS) while on therapy. Five of the seven were associated with TB and 2 with both TB and cryptococcal meningitis. Patients who developed IRIS had a lower median baseline CD4 count (43 [IQR 37-141] cells/mm^3, n=7) than those who did not (141 [IQR 66-196] cells/mm^3, n=250, $p=0.06$).

Eleven patients (4.3%) met numeric WHO criteria[37] for immunological treatment failure: 6 in whom the CD4 count at 12 months was less than 100, and five in whom the final CD4 count was lower at 12 months than at initiation. Four of these had received treatment for active TB during the period of ART. However, in all cases treatment had been successfully terminated at least 5 months prior to the final CD4 estimation, and the low CD4 count cannot be ascribed to a TB-associated reduction in CD4 count. All 11 therefore represent true immunological treatment failure. We were unable to show a significant difference in the extent of weight gain ($p=0.90$) or haemoglobin rise ($p=0.16$) between those with a satisfactory immunological response and those without.

Increases in the CD4 count were not significantly lower for patients with an incomplete virological response in comparison with those with a complete response ($p=0.13$). While the final CD4 count was lower in those who did not achieve a complete virological response (272 [IQR 201-408] cells /mm^3, n=38) versus those who did (332 [IQR 242-431] cells/mm^3, n=219) the result is not significant ($p=0.09$).

Patients treated with tenofovir had a significantly higher CD4 count at baseline (148 [IQR 69-199] cells/mm^3, n=186) than patients who received stavudine (113 [IQR 48-180], n=60) or zidovudine (91 [IQR 50-203], n=11) ($p=0.05$), probably representing the later introduction of tenofovir into the ART coincidental with initiation of patients on ART at higher CD4 counts. The increase in CD4 count across the three drugs did not differ significantly ($p=0.7$) and the post-treatment CD4 count ($p=0.85$) did not differ significantly between the three drugs.

Patients on nevirapine showed a significantly greater increase in CD4 count with a median

increase of 210 cells/mm^3 [IQR 152-277], n=109) than those on efavirenz (157 [IQR 116-248], cells/mm^3, n=148) (p=0.001). Patients treated with nevirapine reached a higher post-treatment CD4 count (285 [IQR 202-383] cells/mm^3) than those on efavirenz (210 [IQR 152-277] cells/mm^3, p<0.0001). Though the baseline CD4 count was lower in patients treated with efavirenz (median CD4 count 114 [40-184] cells/mm^3) than in patients treated with nevirapine (168 [IQR 124-199] cells/mm^3, p<0.0001); two-way factorial ANCOVA indicates that the contribution of nevirapine to a higher eventual CD4 count is significant even after controlling for the baseline CD4 count (p=0.015).

DISCUSSION

ART is a highly effective treatment for HIV-infected individuals which routinely improves both life expectancy and the quality of life in those patients who maintain adequate viral suppression[38,39]. An additional expected benefit is the significant reduction in viral transmission, a recent publication has shown that there is a 93% reduction in risk of infection of a link partner in subjects receiving early ART rather than delayed ART in the developing world[40]; and high rates of protection have been shown, as well as a reduction in mortality, had been shown in a deprived rural area of KwaZulu-Natal[41].

A feature of our patient cohort is the advanced degree of immunodeficiency at the time of ART initiation, with almost all patients having enrolled with a CD4 count less than 200 cells/mm^3. 34% had experienced opportunistic infections prior to enrolment, including a high incidence of pulmonary TB in addition to the classic extrapulmonary TB, disseminated TB and other AIDS-defining illnesses such as cryptococcal meningitis and oesophageal candidiasis. This is still a common experience in resource-limited countries[42]. The average age of our patient cohort was 37 years, which corresponds with the peak of the sexually active population of 15-49.

Fewer males present for ART at an early stage of infection. Male sex has been found to be a significant determinant of a late presentation in Durban[43], in sub-Saharan Africa and in developing countries more generally[44]. Males typically present with advanced disease; there is some evidence that the gap may be increasing even as the proportion of patients presenting at an advanced stage for initiation of therapy decreases[42]. This is reflected in our cohort where the baseline CD4 count was significantly lower for males than for females.

As with other studies from resource-limited settings, the results of our study are encouraging[42]. There is a significant mean increase in CD4 count of 188 cells/mm^3 after 12 months, and this was associated with gains in weight and haemoglobin suggesting an improvement in general health. Only 11 of 257 study patients met the WHO criteria for immunological treatment failure, indicating a 96% immunological response rate. A successful outcome was independent of age, sex, initial viral load (measured in some but not all participants), the baseline CD4 count and the presence of preceding opportunistic infections or of treatment regimen. Interestingly however, patients with immunological failure were no less likely to gain weight or show an increase in haemoglobin. We were unable to show a correlation and the extent of the increase in CD4 count with age or sex in contrast to the findings of Nash *et al.* [42]. We did however find a highly significant correlation between the CD4 count reached at 12 months and the baseline CD4 count. This is in line with the findings of Nash *et al.* in a multinational study, who found that this differentiation persists with time, and that those who start treatment with a lower CD4 count remain at a persistent disadvantage.

Our results also show that the change in recommended regimen from stavudine, with its multiple metabolic complications, to tenofovir, which has a much lower toxicity profile, was not accompanied by any decrease in efficacy, thus confirming previous findings[45]. Our results do however suggest that nevirapine may be more effective in increasing the CD4 count than is efavirenz. This is unexpected, since previous studies in Ethiopia[46] and Thailand[47] have suggested comparable responses for efavirenz and nevirapine. The use of nevirapine is now discouraged in South Africa, given its toxicity profile. Were our observations to be confirmed in further studies however, we may have to consider the possibility that the shift from nevirapine is accompanied by some loss in efficacy.

The findings of this analysis are strengthened by the relatively homogeneous study population receiving treatment at a single facility using standardised clinical protocols. Patients were all ART-naïve and received a standard triple-drug regime with uniform follow-up time points. Quality-assured laboratory assays were all performed in a single nationally accredited laboratory. Our patient population was treated under the government ART roll-out programme and data are therefore likely to be generalizable to other ART programmes in sub-Saharan Africa.

A limitation of our study is that we only followed patients who demonstrated virological

suppression. We are therefore unable to comment on that cohort of patients who failed to respond to ART. We limited our analysis of CD4 cell recovery to the first 12 months of ART; long-term outcomes have not been determined. Nor have we studied the effects of parameters such as genetic determinants (e.g. the protective Δ32 CCR5 genotype) on CD4 response curves. Though our results suggest a greater effect on CD4 count for nevirapine than efavirenz, this is a finding which is best confirmed in a proper randomised controlled trial.

Conclusion

Patients treated in the South African public health sector may be regarded as disadvantaged in comparison with many other groups who have been studied, in that they have, at least until recently, been enrolled on treatment when already significantly immunosuppressed, and with a high likelihood of already having developed opportunistic infections[44]. A recent study from Johannesburg found that patients whose ART was initiated in a private clinic were significantly advantaged in multiple respects in comparison with those who received public sector care[48]. Recent changes in the national guidelines mandating initiation of therapy at a much earlier stage of infection are likely to improve this situation significantly[34,49,50]. Despite this disadvantage however, our results are encouraging given the very high rate of immunological response in our subjects. Our findings support the belief that initiation of therapy at lower CD4 counts disadvantages patients. We showed a correlation between a low baseline CD4 count and the development of IRIS which approached statistical significance: the numbers were small and it is possible that a larger study would confirm this association. Furthermore, we have shown strong correlation between the CD4 count at 12 months and the baseline CD4 counts; recent evidence suggests that the lower CD4 counts achieved by patients who begin therapy late will persist in the medium and long-term[44].

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TABLE 1

Characteristics at baseline. Values are expressed as mean (SD) or median [IQR]. Some patients had more than one opportunistic infection.

	Total	**Male**	**Female**	***p***
N	257	71 (27.6%)	186 (72.4%)	0.001
Age (years)	36.6 (SD 9.3)	36.6 (SD 9.0)	36.7 (SD 9.5)	0.94
Baseline CD4 (cells/mm^3)	139 [IQR 63-195]	90 [IQR 24-180]	148 [IQR 88-199]	0.0003
Opportunistic infections (percentage of the total)	87 (34%)	37 (52%)	50 (27%)	0.001

TABLE 2

Opportunistic infections noted prior to initiation of ART. 14 of the subjects had dual infection: TB and cryptococcal meningitis (9) and TB and candidiasis (2).

Infection	N
Total	87 (33.9%)
Cryptococcal meningitis	17 (6.6%)
Tuberculosis (pulmonary and extrapulmonary)	68 (26.4%)
Pulmonary tuberculosis	44 (17.1%)
Extrapulmonary tuberculosis	17 (6.6%)
Disseminated tuberculosis	7 (2.7%)
Oesophageal candidiasis	13 (5.0%)

TABLE 3

Response to ART at 12 months. Values are expressed as mean (SD) or median [IQR].

	Baseline	**12 months**	***p***
CD4 (cells /mm^3) (n=257)	139 [IQR 63-195]	327 [IQR 235-427]	<0.0001
Weight (kg) (n=253)	67.3 (SD 15.1)	71.4 (SD 15.4)	<0.0001
Haemoglobin (g/dL) (n=244)	11.1 (SD 2.0)	11.9 (SD 1.9)	<0.0001

TABLE 4

Response to ART at 12 months. Values are expressed as mean (SD) or median [IQR].

	Male	**Female**	***p***
Increase in CD4 count	153 [IQR 115-223]	196 [IQR 131-278]	0.01
Increase in weight	3.8 (SD 6.2)	4.1 (SD 6.7)	0. 69
Increase in haemoglobin concentration	0.79 (SD 1.7)	0.77 (SD 1.5)	0.94

TABLE 5

CD4 change by CD4 count strata. Values are expressed as median [IQR].

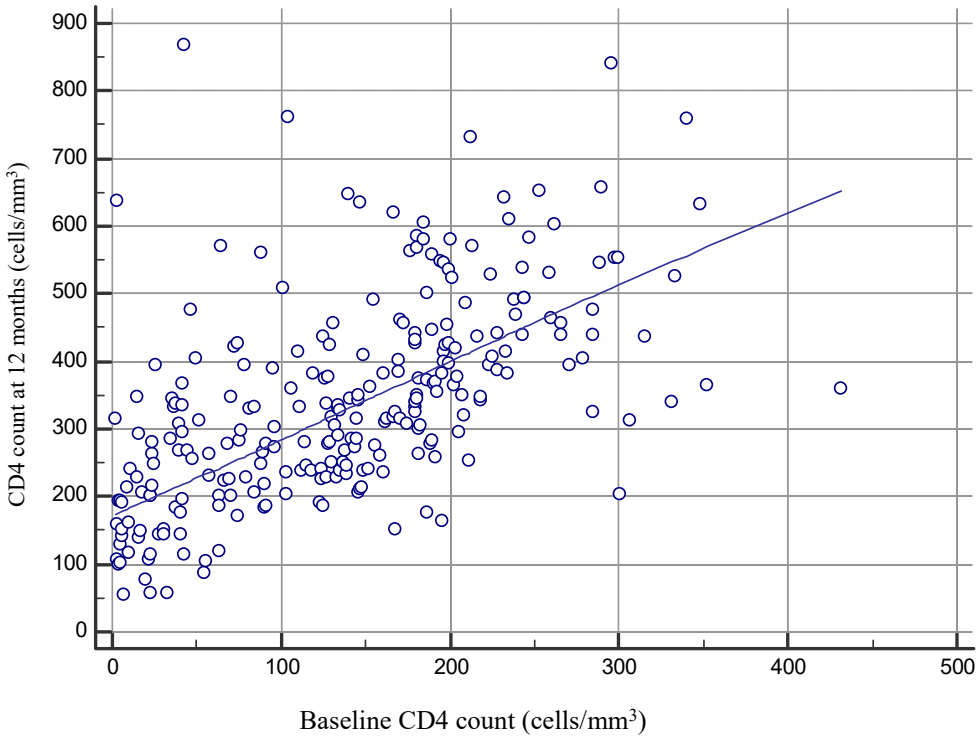
CD4 Group	N	Increment in CD4 count
<50	56	186 IQR [IQR 118-254)]
50-99	35	176 [IQR 128-257]
100-199	110	175 [IQR 119-254]
200-349	54	208 [IQR 149-296]
≥350	2	-31 [IQR -73-12]

FIGURE LEGENDS

Figure 1

There is a highly significant correlation between the CD4 count noted at the time of initiation of ART and that noted after 12 months (Spearman's rho=0.643, $p<0.0001$).

FIGURE 1


CD4 count at 12 months (cells/mm3)
900
800
700
600
500
400
300
200
100
0
0
100
200
300
400
500
Baseline CD4 count (cells/mm3)

APPENDICES

APPENDIX 1

Approved protocol

Dr Mfundo F Mathenjwa

Student number: 211559005

MMed Study Protocol

‘CD4 Count trends in patients initiated on Antiretroviral Therapy’

Internal Medicine

University of KwaZulu Natal

Medical School

January 2013

STUDY QUESTION

To investigate the rate of change of the CD4 count after commencing antiretroviral therapy(ART).

BACKGROUND

The CD4 count and viral load (RNA level) are closely linked to human immunodeficiency virus (HIV)-related illness and mortality. They provide prognostic information on HIV progression and on response to therapy.

CD4 lymphocyte count

The CD4+ T lymphocyte cells are the primary target of HIV. The CD4 count reflects the extent of immune suppression. It is calculated from the CD4 cell percentage and total white blood cell count. The normal values for the CD4 count vary considerably among different laboratories. The mean normal value for most laboratories is approximately 500-1,300 cells/µL. In persons with untreated HIV infection, the CD4 count declines by approximately 50-80 cells/µL per year on average. The pattern of decline is usually slow and steady, taking on average 10 years for a newly infected person to progress to acquired immunodeficiency syndrome (AIDS). There is however great variation among individuals. For some patients, disease progression occurs within a couple of years (rapid progressors) (Anzala *et al.*, 1995). For others, it may take more than 20 years (slow progressors) (Rhodes *et al.*, 2000).

Among those with HIV infection, the CD4 lymphocyte count is the major indicator of immunodeficiency, a main factor in deciding whether to initiate antiretroviral therapy (ART), and an important parameter in monitoring treatment response (Joint United Nations Programme on HIV/AIDS (UNAIDS), 2010). Failure to restore a normal CD4 count following ART is associated with increased morbidity and mortality(Thompson *et al.*, 2010).

Prophylaxis against opportunistic infections is based on CD4 count, thus a CD4 count of less than 200 cells/µL is an indication for prophylaxis against *Pneumocystis jiroveci* pneumonia. The CD4 count also guides decision making in determining when to stop prophylaxis against opportunistic infections in patients whose CD4 counts respond to ART. For monitoring purposes, the CD4 count should be repeated approximately every 3 to 6 months both in stable untreated patients and in patients on ART. It should however be checked more frequently in ART failure, in the face of a rapidly declining CD4 count and when switching therapy. In our local resource-limited setting, however, CD4 count is done yearly.

Viral load

The HIV-1 viral load measurement indicates the number of copies of HIV-1 RNA per millilitre of plasma. Although HIV ultimately resides within cells, the plasma measurement is an accurate reflection of the burden of infection and the magnitude of viral replication. It is critical in monitoring virologic response to therapy.

Because CD4 and clinical responses may lag behind changes in viral load, viral load testing is essential for detecting virologic failure in a timely manner. With an effective ART regimen, suppression to undetectable levels should be achieved within 3 to 6 months after initiation of therapy(Kaplan *et al.*, 2009).

ART in South Africa

South Africa has the highest HIV prevalence in the world, and additionally has the largest ART program(Lawn *et al.*, 2009). From 2004 antiretroviral therapy was made available to all public sector adult patients with a CD4 count of less than 200 cells per µL and those with Stage 4 clinical disease.

In 2010 the program began to enrol pregnant and tuberculosis-co-infected subjects with a CD4 count of less than 350 cells/ml. More recently, in August 2011, the South African public health sector has implemented enrolment of all patients with a CD4 count of less than 350 cells/µL regardless of concurrent tuberculosis or pregnancy or clinical stage, as suggested in the World Health Organisation HIV Guidelines(World Health Organization, 2009). This will significantly reduce long term morbidity and mortality in the HIV-infected population though at the expense of an immediate financial strain.

A typical first-line South African antiretroviral therapy regimen includes two nucleoside reverse transcriptase inhibitors (NRTIs) and one non-nucleoside reverse transcriptase inhibitor (NNRTI). Failure of this regimen may require switching to a protease inhibitor (PI)-containing second line regimen. Patients with a CD4 count more than 350 are screened for opportunistic infections and AIDS defining illnesses. If none are found, the patient returns after 6 months for a repeat CD4 count. Patients at entry level are screened for hepatitis B, syphilis and active tuberculosis . Baseline CD4 cell count and biochemical profile including full blood count, renal and liver function tests are performed. Follow up is subsequently every 12 months for clinical and biochemical review. This entails screening for opportunistic infections and drug adverse effects. Of paramount importance is the 12 monthly monitoring of immunologic and virologic response, which involves monitoring of the CD4 count and viral load.

A persistently low CD4 count and high HIV viral load in a compliant patient raises a question of viral resistance to therapy. Twelve months after initiation of antiretroviral therapy the viral load should be undetectable. However there are no strict guidelines stipulating the expected average increase in the CD4 count, below which one would be classified as having treatment failure. A number of definitions of treatment failure, or suboptimal immune

response, have been used. Suboptimal reconstitution has been defined as: an increase in CD4 count of less than 200 cells/mm^3, or failure to attain an absolute CD4 count exceeding 200 cells/mm (Hermans *et al.*, 2010), failure to develop a more than 30% increase in CD4 count or an absolute CD4 less than 200 cells/mm^3 with full suppression of HIV replication(Gazzola *et al.*, 2009) during the first 6–12 months of ART (Lifson *et al.*, 2011). This is partly because ART-induced increases in CD4 cell count are much more variable from patient to patient than viral load. In multivariate analysis adjusting for baseline CD4 and post-ART time interval, CD4 responses were lower in patients who met the following criteria: longer time from HIV seroconversion to initiation of ART, lower pre-ART CD4 nadir, higher pre-ART viral load and clinical AIDS before ART($P<0.05$)(Lifson *et al.*, 2011). This study also demonstrated that the greatest CD4 changes occurred in the first 6 months post-ART (with an average increase of 93-151 cells over baseline values), with lesser increases through the first four years (average increase of 22-36 cells per year).

Four other studies worthy of mention have demonstrated variable responses in CD4 count after a year on ART. A study performed on 596 ART-naïve individuals in Cape Town, South Africa, demonstrated a increase from a median CD4 cell count of 97 cells/µL to 261 cell/ µL over 48 weeks (Lawn *et al.*, 2006).

In a pan-European observational study on 1835 ART-naïve individuals, the greatest mean yearly increase in CD4 count of 100 cells per µL was seen in the year after starting ART(Mocroft *et al.*, 2007). In the Swiss-HIV Cohort Study, median CD4 T-cell increases following viral load suppression were 87 cells/ µL within 1 year of initiation of ART. This study also showed that median CD4 T-cell increases were higher for female gender, lower age and a higher viral load at ART start. Patients on tenofovir showed significantly lower increases compared with stavudine (Wolbers *et al.*, 2007). In a study done in Botswana, on 633 patients initiated on an non-nucleoside reverse transcriptase inhibitor-based combination of ART , the median CD4 increase was 169 cells/µL at 1 year(Hermans *et al.*, 2010).

In other studies to evaluate prior ART experience and host characteristics as determinants of immunologic and virologic response to ART, better short- and long-term CD4 cell responses were observed in treatment-naive users. Intermittently and consistently experienced users did not significantly differ in response. Whereas race did not affect response, among those initiating ART with $>400\times10^6$ CD4 cells/l, younger age and the Δ32 CCR5 genotype were associated with a better short term CD4 cell response (Yamashita *et al.*, 2001). It has been suggested that zidovudine-containing regimens and male sex are also associated with lower responses (Hermans *et al.*, 2010).

It is also noteworthy that concurrent opportunistic infections, the commonest being tuberculosis, would be expected to blunt ART induced CD4 count improvement. In a study in Uganda , patients who developed tuberculosis in the first 24 months post-inception of HAART had sub-optimal immunologic recovery. Multiple linear regression analysis of the

factors associated with CD4 change at two years confirmed that incident tuberculosis during ART was associated with lower CD4 count recovery (Hermans *et al.*, 2010).It would be of much clinical value to study the trend in CD4 count in response to ART in a South African setting. The aim is to incorporate the CD4 response as a guide in defining ART treatment failure. Using CD4 count as a tool is more feasible in our resource-limited setting as viral RNA is only done at the central virological laboratories, resulting in delayed turn-around times for results, and is additionally costly. On the other hand CD4 count analysers are more accessible as they are available even at district level hospitals. This study will also emphasize the importance of identifying immunological success in addition to virological suppression and optimizing treatment for immununological failure.

AIM OF STUDY

To investigate the rate of change in CD4 count over 12 months in patients recently initiated on ART.

OBJECTIVES

1. Assess CD4 and viral load response after the first 12 months of antiretroviral therapy.
2. To investigate the impact of age, baseline CD4, HIV clinical stage, intercurrent infection(including tuberculosis) and concurrent non-ART medication on immunologic response after 12 months of ART.

DESIGN AND METHODS

A retrospective chart review of patients enrolled at the Philani Clinic (the HIV clinic attached to King Edward VIII Hospital) will be carried out. This will comprise 250 patients enrolled for the first time on the ART programme at the clinic after January 2010. A record of their age, sex, baseline CD4 count, and regimen type will be documented at initiation and at 12 months post-initiation of ART. The presence of HIV related Stage 3 or 4 illnesses, and intercurrent infections that occur within 2 months prior to initiation of ART and within the 12 months study period will be documented. This will include patients with tuberculosis(TB) and those who develop Immmune Reconstitution Inflammmatory Syndrome(IRIS) post-initiation of ART.The variable of interest, which is, the change in CD4 count will be monitored and correlated with the viral load trend.

Data will be analysed for correlations with demographic data (age, sex and race), presence of intercurrent infection(including TB), WHO HIV clinical stage, baseline CD4, ART adherence, and use of other drugs known to interact with ART. Non-adherence will be

defined as missing any medication refill during the 12 months study period according to pharmacy records. This is with the assumption that patients who collect their medicine refill do take their pills at home.

Inclusion criteria

- Patients 18 years and older, commenced for the first time on ART from January 2010, who were subjected to clinical review, and immunologic and virologic monitoring 12 months after initiation of ART.

Exclusion criteria

- Patients with no data at 12 months
- Documented psychiatric illness sufficient to alter expected treatment adherence.
- Patients with documented poor adherence

Sample size

250 sequential patient clinical charts will be reviewed.

Limitations of study

1. Lack of physical patient interviews to assess adherence directly.

2. Lack of means to assess the effect of genetic determinants (e.g. the protective Δ32 CCR5 genotype) on CD4 response curves.

3. Inavailability of baseline viral load , as this not performed in the public sector

Data: Management and statistical analysis

Patient data will be entered into a spreadsheet. Data will be analysed according to age,race, sex, baseline CD4, the presence of intercurrent AIDS-related illnesses(other than tuberculosis), ART regimen and use of non-ART medication. Mean change in CD4 countwill be documented, and statistical significance and confidence intervals determined where appropriate.

Ethical considerations

Specific consent will not be obtained from patients since this is a retrospective review. Data will be anonymised. Approval will be obtained from the UKZN Biomedical Research Ethics Committee.

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