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# Impact of Highly Active Anti-Retroviral Therapy on paediatric Human Immuno-Deficiency Virus associated left ventricular dysfunction within the Johannesburg teaching hospital complex

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## Chapter 1: Literature Review

### 1.1 Introduction

The combination of the Human Immuno-deficiency Virus (HIV) and Acquired Immuno-deficiency syndrome (AIDS) results in a multi-system disease involving any of the organ systems of the body. One of the more serious complications of HIV infection is cardiovascular system dysfunction in the form of left ventricular myopathy which may be secondary to myocarditis or cardiomyopathy. The median survival of such patients is markedly reduced compared to patients with normal cardiovascular evaluation assessed echocardiographically at a comparable infection stage.[1,2] The cardiovascular manifestations are seen with advanced disease which may be associated with severe weight loss or wasting disease, HIV-associated encephalopathy, very low CD4 counts, and exceedingly high viral loads.[3-5]

Other cardiovascular manifestations of HIV infection include pericardial disease, infective endocarditis, rhythm disturbances, cor pulmonale, vasculitides, cardiac malignancies, and congenital heart defects which have been reported to be as high as 5% compared to the general population incidence report of 0.8%.[3,6-9] There are no studies though, that have looked at cardiac teratogenicity of HIV. Although these cardiovascular manifestations of HIV/AIDS and their poor outcomes are well documented in adults, [2,4,5,7,10-12] there is paucity of similar information assessing cardiac outcomes in children with HIV in the pre-Highly Active Antiretroviral Therapy (HAART) and post HAART era. One study completed over a two year period showed that 10% of HIV infected children who did not receive HAART developed congestive heart failure and approximately 20% developed LV dysfunction or

dilatation.[6] A literature search using the Pubmed Internet database of Journal References, revealed four studies that assessed the outcome of left ventricular dysfunction and cardiomyopathy in HIV infected children receiving HAART.[13-16] All four studies documented improvement of the left ventricular function in patients receiving combination therapy compared to those who received monotherapy or those who did not receive any antiretroviral treatment. The improvement in LV function was noted as early as six months, to as late as six years following initiation of anti-retroviral therapy.[13-16] The anti-retroviral drugs administered in these studies included various combinations of Zidovudine, Didanosine, Stavudine, Lamivudine, Nelfinavir and Ritonavir. None of the patients enrolled in the 4 studies developed cardiomyopathy following the administration of Zidovudine and Didanosine,[13-16]both of which have been associated with skeletal muscle and myocardial dysfunction.[17] The association of antiretroviral drugs with myocardial dysfunction has important implications in the anti-retroviral drug therapy choices for children that have myocardial dysfunction prior to starting therapy or those developing myocardial dysfunction following the commencement of anti-retroviral therapy.

**1.2 Human Immuno-deficiency Associated Myocardial Dysfunction**

Cardiac muscle disease is an important complication of HIV infection and manifests as myocarditis, dilated cardiomyopathy, and isolated left or right ventricular dysfunction.[18]

Left ventricular dysfunction is an important predictor of overall mortality, even after adjustment for age, height, CD4 cell count, and progressive neurological disease.[19]

A discussion of the various causes of LV dysfunction follows.

### 1.2.1 Sub-clinical Left Ventricular Dysfunction

Based on echocardiography imaging using 2 Dimensional, M-Mode and Pulse Wave Doppler; sub-clinical LV dysfunction is defined as either systolic or diastolic dysfunction according to the following parameters:

1) Systolic:

Elevated LV diameters in end diastole and end systole, interventricular septum and LV posterior wall thickness in diastole and systole.[20] The ejection fraction and fractional shortening are within normal in patients with sub-clinical LV dysfunction.[20]

2) Diastolic:

Deranged measurements of peak velocity of early (E wave) and late (A wave) mitral outflow and the E/A ratio, mitral deceleration time, and the isovolumic relaxation time (IVRT) defined as the time interval between aortic valve closure and mitral valve opening.[20] Abnormal echocardiographic findings may be recorded in asymptomatic HIV infected patients, either as diastolic dysfunction or systolic dysfunction,[21,22] which are reversible if cardiac failure therapy is initiated early. However, if undiagnosed and untreated, sub-clinical left ventricular dysfunction may be associated with fatal outcomes. [21,22] The aetiology is thought to be due to myocarditis, but in many cases is unknown.

### 1.2.2 Myocarditis

The Dallas criteria until recently was the gold standard used to diagnose myocarditis. The criteria incorporate the histological presence of an inflammatory infiltration of the myocardium, with adjacent myocyte necrosis or degeneration that is not typical of ischemic damage associated with coronary artery disease.[23]

Three different histopathological patterns of myocarditis have been described in HIV infected patients, viz:

i) Lymphocytic infiltration with myocyte necrosis which meet the Dallas criteria;[24]

ii) Lymphocytic infiltration without inflammation,[25] and

iii) Myocytic damage without evidence of inflammatory infiltrate.[26]

The prevalence of myocarditis in HIV infected adult patients ranges from 6% to 52%.[27,28] The aetiology is thought to be related to various factors including direct HIV myocardial invasion, autoimmune processes, opportunistic infections, cytokine release, autonomic dysfunction, nutritional deficiencies, drugs and cardiotoxins like alcohol and cocaine, endothelial dysfunction and myocardial infiltration by AIDS-associated malignancies.[29-39]

Direct myocardial invasion by HIV itself was shown by isolation of the virus in a myocardial biopsy sample taken from an adult patient with AIDS and dilated cardiomyopathy.[32] Other instances of myocardial invasion by HIV have been reported using in-situ hybridization testing on the myocardium of HIV infected patients which reveal the presence of HIV nucleic acid sequences.[33] Even though the cardiac myocytes themselves lack the CD4 receptors, several mechanisms for myocyte damage by the HIV virus have been suggested. The virus has been demonstrated to infiltrate the myocardial interstial space where it is harboured by macrophages and dendritic cells which possess CD4 receptors.[34] Other methods of incorporation into the myocyte have been shown through in-vitro studies whereby newly developed human foetal cardiac myocyte cell lines ingest HIV-1 through a specific Fc receptor.[35]

One of the hypotheses of the mechanism of myocardial damage caused by HIV is that HIV gp120 surface antigen attaches to the HIV gp120 antigen receptor on the cardiac myocyte, leading to activation of the enzyme p38 mitogen activated protein (MAP) kinase, which results in activation of the inflammatory cascade, with the release of tumour necrosis factor alpha and cytokines leading to myocardial destruction and dysfunction.[36,37] A study using immunohistochemical methods on adult rat ventricular myocytes has demonstrated the presence of a CXCR4 myocardial receptor, which allows for binding of the HIV gp120 antigen.[38]

Affected reservoir myocardial interstitial dendritic cells have been implicated in the release of cytokines such as tumour necrosis factor alpha, interleukin-1, interleukin-6 and interleukin-10, which lead to progressive myocardial damage followed by reduced myocardial function.[34,40] Another mechanism of myocytic damage may be the result of ‘’innocent bystander destruction”, whereby toxic enzymes and cytokines are generated in the interstitium by the HIV replication process.[39]

Many other causes of myocarditis in HIV infected patients have been suggested and include autonomic dysfunction, HIV malignancies, nutritional deficiencies, HAART, and a variety of opportunistic infections which have been identified in 10-15% of cases (table 1.1).[36]

**Table 1.1 Causes of myocarditis in HIV infection**[36]

| | |
|---|---|
| *Bacterial* | Mycobacterium tuberculosis |
| | Mycobacterium avium-intracellulare |
| | Staphylococcus aureus |
| *Viruses* | HIV |
| | Cytomegalovirus |
| | Herpes simplex |
| | Coxsackie |
| *Protozoa* | Toxoplasma gondii |
| | Pneumocystis carinii |
| | Microsporidium |
| *Fungi* | Cryptococcus neoformans |
| | Histoplasma capsulatum |
| | Aspergillus fumigatus |
| | Candida species |
| | Coccidioides immitis |

### 1.2.3 Dilated Cardiomyopathy

The initial case reports of HIV associated dilated cardiomyopathy in adult patients were documented by Cohen IS et al, in 1986.[41] HIV associated cardiomyopathy is usually associated with advanced immunosuppression, with very low CD4 cell counts, and is independently associated with death.[42,43] The prevalence of HIV associated cardiomyopathy in the western world in the pre-HAART era in adults was reported to be 30-40%,[44] whereas in Africa, the prevalence ranges between 9% and 57%.[42] Myocarditis has been shown to be the main cause of dilated cardiomyopathy in patients with HIV (Table 1.2).[4,45] One study demonstrated myocarditis to be the underlying cause of dilated cardiomyopathy in 80% of adult patients with HIV. [46]

**Table 1.2 Possible causes and links to HIV associated Dilated Cardiomyopathy[4,45,46]**

| |
|---|
| ***Infectious*** |
| HIV, Toxoplasma gondii, coxsackievirus group B, |
| Epstein–Barr virus, cytomegalovirus, adenovirus |
| ***Autoimmune response to infection*** |
| ***Drug related*** |
| Cocaine, nucleoside analogues, IL-2, |
| doxorubicin, interferon |
| ***Nutritional deficiency/wasting*** |
| selenium, B12, carnitine |
| ***Metabolic/Endocrine*** |

Thyroid hormone, growth hormone

Adrenal insufficiency, hyperinsulinemia

***Cytokines***

TNF-alpha, nitric oxide, TGF-beta, endothelin-1

***Miscellaneous***

Hypothermia

Hyperthermia

Autonomic insufficiency

Encephalopathy

Acquired immunodeficiency

HIV viral load, length of immunosuppression

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**1.3 The Immune Status and Myocardial Dysfunction in HIV infection.**

HIV associated cardiovascular complications are usually documented in adult patients with advanced disease which is associated with low CD4 cell counts, high viral loads and co-existent encephalopathy.[36,47] Patients with HIV and established LV systolic and diastolic dysfunction inevitably develop rapid clinical deterioration which is associated with an equally rapid decrease in CD4 count.[47] It follows therefore that a pre-emptive echocardiographic assessment of the left ventricular function should be undertaken in patients with low CD4 counts.[4,36,46,49]

The introduction of HAART leads to a reduction in viral load and an increase in CD4 cell count.[45] The improvement of the immune system in turn leads to a decrease in opportunistic cardiac infections followed by a decrease in the incidence of cardiovascular complications associated with HIV infection.[4,11]

### 1.4 Nutritional Deficiencies and Cardiovascular Dysfunction in HIV Infection

Reduced cardiac performance, hypotension and bradycardia have been associated with malnutrition in both human and animal subjects.[51,52] Adult patients with advanced HIV and nutritional deficiencies have also been shown to have associated left ventricular dysfunction.[36] Malnutrition is a common manifestation in paediatric patients with HIV infection and is thought to be secondary to increased metabolic demands, insufficient energy substrates, malabsorption and diarrheal diseases which result in electrolyte, trace element and vitamin losses.[53]

Selenium deficiency in particular has been associated with myocardial dysfunction in HIV infected adults which is reversible with selenium supplements. [36,54]Malnutrition is associated with suppression of the immune system which further aggravates an already compromised immune system in HIV infected patients. A marked improvement in nutritional status, growth with height gain, immune function as well as left ventricular performance has been seen with the introduction of HAART.[55,56]

### 1.5 Introduction of HAART in the HIV Infected patient with Myocardial Dysfunction

Treatment of HIV infected patients with HAART has been shown to not only improve survival but also contributes to better quality of life. Of great importance is the benefit to patients with associated cardiomyopathy. The prevalence of cardiomyopathy in HIV infected

patients has been reduced dramatically by the introduction of HAART. The reduction, compared to the pre HAART era, has been reported to be almost seven fold in one adult patients' study [11] and to be 30% in another.[55]

As was mentioned in the introduction, there are only four studies (Pubmed Search) documenting the outcome of myocardial dysfunction in children with HIV following treatment with anti-retroviral medications. [13-16] There are no studies from Africa that have analyzed the outcome of left ventricular dysfunction secondary to cardiomyopathy or myocarditis in the paediatric age group treated with HAART.

Interestingly, some of the Nucleoside Reverse Transcriptase Inhibitors (NRTIs) group of medications used in some of the HAART regimes paradoxically cause cardiomyopathy by damaging the myocardial and skeletal mitochondria which manifests with lactic acidosis, skeletal and cardiac myopathy, amongst other complications.[57,58]

Another cardiovascular related complication of HAART is the Metabolic syndrome associated with Protease Inhibitors (PI's) and to a lesser extent, NRTI's as shown in one study in children.[59] These patients present with dyslipidemia, acute pancreatitis, lipodystrophy or lipoatrophy, glucose intolerance, and systemic hypertension which may result in coronary artery disease and cerebro-vascular accidents.

### 1.5.1 HAART associated Cardiomyopathy due to Mitochondrial Toxicity

In the early 1990s, when complications of anti-retroviral (ARV) treatment in adult patients with HIV were being documented, Zidovudine and Didanosine (NRTI's which are associated with mitochondrial toxicity), were shown to predispose to dilated cardiomyopathy.[60,61] The toxicity results from either mitochondrial DNA (mtDNA) deletion or depletion.[59] The NRTI's

cause depletion and mutation of mtDNA by inhibiting human DNA polymerase gamma, resulting in reduced synthesis of mtDNA-encoded protein sub-units that are needed for oxidative phosphorylation. Consequently there is failure of generation of ATP and increased production of oxygen radicals which leads to further mtDNA damage.[62] NRTI's are transported and phosphorylated into their active form within the mitochondrion. The medications also compete with the natural nucleotide pool involved in mtDNA replication, resulting in reduced mtDNA production.[63]

There is a combination of factors that cause certain NRTI-exposed patients to develop mitochondrial toxicity,[63,64] such as genetic predisposition, duration, and type of NRTI exposure. The various NRTI's differ in their ability to induce mitochondrial toxicity.[65] Zalcitabine, Stavudine and Didanosine have the greatest mitochondrial toxicity effect, followed by Zidovudine, with Lamivudine, Abacavir and Tenofovir having the least effect.[66] It has been shown that mtDNA mutations in patients receiving HAART accumulate over time. Although there are mtDNA repair mechanisms in place they are less effective than nuclear DNA repair mechanisms.[67]

Mitochondrial toxicity may be exacerbated by using two or more NRTI's in the same patient simultaneously.[68] Interestingly, infants exposed to NRTI's as part of prevention of perinatal transmission of HIV infection have not been shown to develop cardiac complications.[69] On the other hand, mitochondrial DNA depletion in Peripheral Blood Mononuclear Cells (PBMC's) of infants exposed to NRTI's perinatally has been shown to persist for up to two years of age.[70]

The management of adult patients with HIV and features of cardiac mitochondrial toxicity involves switching from NRTI's known to have mitochondrial toxicity e.g. Stavudine, to less mitochondrial toxic NRTI's such as Lamivudine or Abacavir. The mitochondrial damage is reversed and results in improved mtDNA production and mitochondrial enzyme activity.[71] NRTI-sparing regimens introduced into adult treatment protocols have been shown to have fewer NRTI-associated mitochondrial toxic effects.[72]

### 1.6 Morbidity and Mortality in Patients with HIV Infection and Myocardial Dysfunction

#### 1.6.1 Pre-HAART Era

Cardiac disease has been documented in paediatric patients with advanced HIV-1 infection.[73] A close relationship between advanced HIV infection, cardiac failure, and encephalopathy which is an AIDS defining manifestation, has been reported.[73] HIV infected children with congestive cardiac failure respond well to anti-failure treatment if started early.[74,75] Symptoms of congestive heart failure may be attributed to respiratory disease, therefore these patients run the risk of misdiagnosis of their cardiac disease with delay in appropriate treatment. Consequently there is an increased risk of morbidity and mortality from cardiac failure, particularly in children with poor growth and those who are underweight for age prior to commencing treatment.[76] Other factors that have been shown to affect survival negatively include suppressed CD4 counts, a history of Zidovudine therapy and *Pneumocystis carinii* (*jiroveci*) pneumonia in patients with decreased baseline fractional shortening (FS), increased end-diastolic and end-systolic dimensions, increased wall thickness, increased heart rate, and increased afterload.[76] Of note, both decreased FS z-scores and increased wall thickness z-scores have been shown to be independent

prognostic risk factors of mortality after adjusting for CD4 count z-scores, HIV viral load, and the presence of encephalopathy. A reduced FS has been shown to be a reliable marker of increased mortality for up to three years before death. In addition, increased LV mass and end-systolic dimensions are also dependable indicators of poor prognosis in the 2 years prior to demise.[76] Chronic cardiac disease as a cause of death has been reported in 11.8% of children dying from an HIV-related disease.[77]

Other studies examining the relationship of cardiac disease and HIV have reported the presence of mild LV dysfunction in 18% of HIV-1 infected children which was associated with a 55.4% 5-year mortality, whereas the presence of increased left ventricular mass was associated with a 5-year mortality of 75%.[79]

### 1.6.2 HAART Era

Although HAART has prolonged survival, improved the quality of life and reduced the incidence of HIV-1 associated cardiovascular complications,[11,55] the treatment itself has been associated with cardiac disease .[48,58] The main complications reported have been myocardial dysfunction caused by NRTI induced mitochondrial toxicity and PI induced dyslipidemia (paragraph 1.5). Many of the metabolic complications associated with PI's have not been documented in paediatric patients receiving PI's and NRTI's simultaneously.[15]

## 1.7 Conclusion

HIV associated cardiovascular disease in the adult and paediatric populations is common and is often seen in advanced disease linked to a low CD4 count, high viral load and encephalopathy. The advent of HAART has led to improved survival, better quality of life and reduction in the incidence of cardiovascular complications, including HIV associated

cardiomyopathy. However, the drugs themselves may have serious cardiovascular side effects due to complications related to mitochondrial toxicity and the metabolic syndrome. The development of newer and safer ARV's continues, and has resulted in less cardiovascular adverse effects, thus giving new hope to patients who live with HIV and are subjected to lifelong ARV therapy.

## Chapter 2. Motivation for Research

The implementation of a national Anti-retroviral treatment roll-out programme in South Africa in 2004 has significantly altered the outcome of paediatric patients with HIV/AIDS.[83-85] The impact of HAART on the outcome of patients with co-existing cardiovascular diseases is poorly documented. The intention of the proposed study is to record the effect of HAART in children infected with HIV presenting with left ventricular dysfunction at an African tertiary care center.

## Chapter 3. Aims and Objectives

The paucity of information regarding the effects of HAART on the heart in children from sub-Saharan Africa where HIV is endemic has prompted the undertaking of this research project. The aim is to assess the outcome of children with HIV and left ventricular dysfunction treated with HAART in an African tertiary care setting. The close association of poor nutritional status, high viral loads and low CD4 counts to cardiac disease in patients with HIV initiated a parallel evaluation of their nutritional status, viral loads and immune response to treatment.

The main aim is to describe the progress of a series of cases that were seen at a cardiac clinic with HIV and LV dysfunction before Highly Active Antiretroviral Therapy (HAART) was

introduced nationally and a group who received HAART at Chris Hani Baragwanath Academic Hospital, Rahima Moosa Hospital, and Charlotte Maxeke Johannesburg Hospital.

The following components will be analysed:

1) Retrospective review of left ventricular fractional shortening on echocardiographic examination pre- and during HAART in patients who were HIV infected and were found to have left ventricular dysfunction.

2) Assessment of the effect of HAART on the immunological system (defined by changes in the CD4 percentage, and viral load), the nutritional system and LV function in HIV infected patients with left ventricular dysfunction.

3) Assessment of LV function over time in patients who were HAART-naïve .

4) Outcome, which will include death, loss to follow up, improvement of FS, CD4 percentage, Viral load count and growth.

## Chapter 4. Study Methods

**Patients:**

The data bases of the Paediatric Cardiology Divisions of Chris Hani Baragwanath Academic Hospital and Charlotte Maxeke Johannesburg Academic Hospital which were established in 1992 and 1994 respectively; patients records in Paediatric Cardiac Clinic at Rahima Moosa Hospital (all of which belong to the University of the Witwatersrand Teaching Hospital Complex in Johannesburg) were searched for patients less than 14 years with HIV and LV dysfunction up to June 2008. The patients who were diagnosed with LV dysfunction were

seen as referrals from the general paediatric wards, transfers from referring hospitals or as referrals from the HIV clinics. These were patients with either cardiac or respiratory symptoms.

Following identification of these subjects, data regarding immunological status, nutritional status and antiretroviral therapy was reviewed from specialized anti-retroviral treatment roll out sites which included the Harriet Shezi Clinic at Chris Hani Baragwanath Academic hospital, the IC2 clinic at Rahima Moosa Hospital, the HIV clinic at Charlotte Maxeke Johannesburg Hospital, the Perinatal HIV Research Unit (PHRU) of University of the Witwatersrand and from the National Health Laboratory Services (for those patients whose records were not found at the listed sites).

The following data was entered on to a data collection sheet (See Appendix 2):

a) Age at first presentation.

b) Sex.

c) Echocardiographical assessment of myocardial function pre- and during HAART therapy.

All patients suspected of having heart disease had echocardiography including M-Mode, 2-Dimensional, Colour Flow Imaging, Continuous and Pulse Wave Doppler in the admission area, general paediatric ward, high care area or cardiac clinic (echocardiography laboratory).

d) List of the anti-retroviral drugs received and the duration of therapy.

The antiretroviral therapy regimen used during the study period is attached as Appendix 1[86]

e) List of heart failure medication.

Following diagnosis of LV dysfunction by echocardiography, the patients were commenced on antifailure medication by their attending physicians.

In the pre-HAART era, patients were treated with Digitalis, diuretics (Furosemide, Spironolactone), Angiotensin Converting Enzyme Inhibitors (ACEI's) and Potassium supplements. In the mid 2000 (coincidentally, at approximately the same time that HAART was rolled-out nationally), Beta blockers (Carvedilol) were introduced as part of therapy for children with dilated cardiomyopathy.

f) Changes in the CD4 percentage and viral load levels during treatment.

g) Trends in weight-for-age and height-for-age z-scores.

h) Final outcome including morbidity, co-morbidity and mortality.

**Statistical Analysis**

Variables such as fractional shortening, CD4 percentage, viral load, weight, height/length pre- and during HAART were compared using a t-test (for normal distribution) and the Mann-Whitely test (for abnormal distribution). A paired t-test was used to compare variables within groups of normal distribution, whereas the Wilcoxon test was employed for comparing non-normal distribution variables.

**Inclusion Criteria**

1) All patients aged less than 14 years with laboratory confirmation of HIV infection viz. HIV ELISA test after the age of 18 months or an HIV DNA PCR test for patients less than the age of 18 months.[86]

2) All patients with left ventricular dysfunction assessed using echocardiography.

3) All patients with recorded nutritional status.

**Exclusion Criteria:**

Patients whose records had deficient information regarding follow up fractional shortening (both groups), immunological data and HAART combinations (HAART group) were excuded.

**Ethical Clearance:** Ethics approval to use data from the three academic institutions was obtained from the Ethics Committee for Research on Human Subjects (Medical) of the University of the Witwatersrand. Permission to use patient data was also obtained from the various hospital authorities. All patient data was analysed confidentially and without prejudice to the patients.

## Chapter 5. Results

### 5.1 Demographics

A total of 71 patients were found to be HIV positive and had an initial fractional shortening of less than 25%. Only 34 patients met the inclusion criteria (Table 5.1). Eighteen patients received HAART (group one) and sixteen were HAART naïve (group two). Excluded from the study were 4 patients from group one and 33 patients from group two because there was lack of information regarding follow up of LV function in both groups and details about HAART regimens and immunological statuses in group one. It was not possible to quantify the percentage of HIV positive patients with LV dysfunction compared to the population of the HIV positive patients as the patients referred to the three Cardiac Clinics originated from the Johannesburg Teaching Hospital Complex as well as other referral clinics and provinces.

The median follow up duration of group one was 21 months (range, 2 weeks to 38 months) while the median follow up period of group 2 was 28 days (range, 2 days to 9 months). The median weight-for-age z-scores and median height-for-age z-scores at baseline are included in Table 5.1. Initial CD4 percentage (median) and viral load log values (median) for group one are also documented in Table 5.1. The median LV fractional shortening at baseline was recorded for both groups.

**Table 5.1. Demographics and Baseline Nutritional, Immunological and Left Ventricular parameters (Median)**

| | Group one (n=18) | Group two(n=16) |
|---|---|---|
| Age (mo) | 29-152 (Median=94) | 4-159 (Median=34) |
| Sex | Males=9<br>Females=9 | Males=10<br>Females=6 |
| HIV Testing | HIV Elisa=16<br>HIV DNA PCR=2 | HIV Elisa=10<br>HIV DNA PCR=6 |
| Inclusion Period | October 2003 to June 2008 | April 2001 to March 2006 |
| Median Follow up Duration | 21mo | 28days |
| Median Baseline WAZ | -1.70 | -2.47 |
| Median Baseline HAZ | -2.16 | -1.16 |
| Median Baseline CD4 | 12% | Not Available |
| Median Baseline VL $Log_{10}$ | 5.4 $Log_{10}$ | Not Available |
| Median Baseline FS | 17% | 13% |

n=number; mo=months; HIV=Human Immunodeficiency Virus; Elisa=Enzyme-linked Immunosorbent Assay; DNA= Deoxyribonucleic Acid; PCR = Polymerase Chain Reaction;

WAZ = Weight-for-age z-scores; HAZ = Height-for-age z-scores; VL = Viral load; FS = Fractional shortening.

### 5.2 Echocardiographical data related to myocardial function pre- and during HAART

The initial FS in group 1 ranged between 7% and 27% (median, 17%). The improvement in FS following HAART was statistically significant and ranged between 10% and 43% (median, 33.5%), (Figure 5.1).

The initial FS in group 2 ranged between 9% and 23% (median, 13%). The FS of seven patients that were followed up ranged between 6% and 34% (median, 19%) over a period of 2 days to 9 months (median, 28 days).

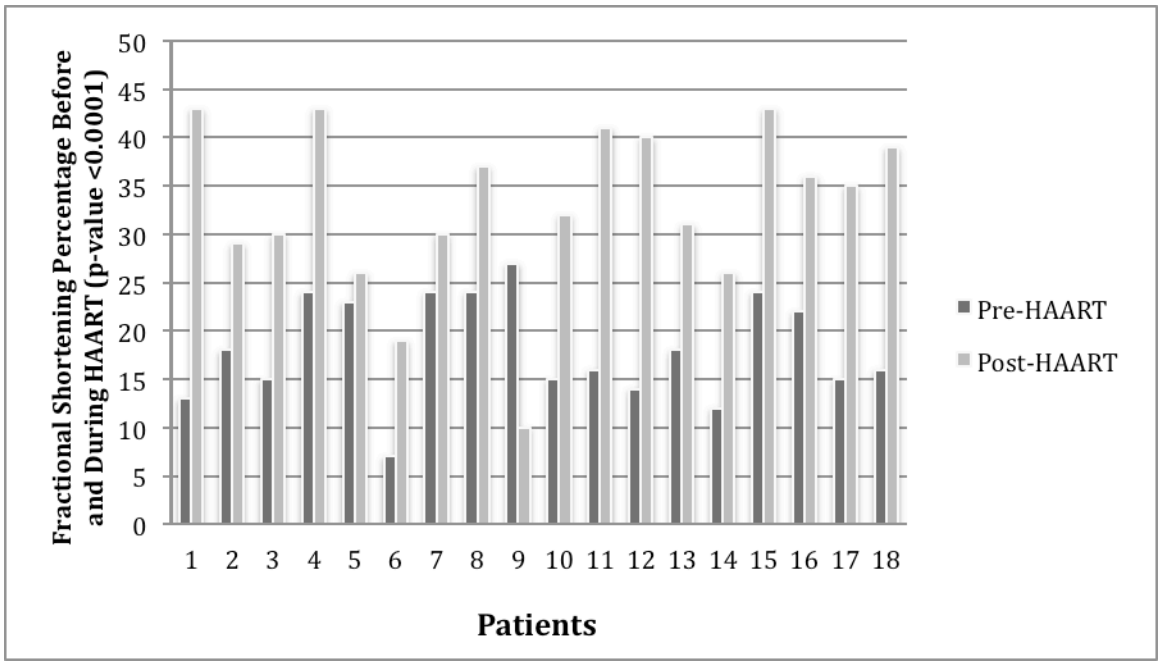


**Figure 5.1. LV Fractional Shortening Pre- and during (post-) HAART (n= 18)**

### 5.3 Cardiac failure medication

Following echocardiographic confirmation of LV dysfunction, all group 1 patients were commenced on antifailure medication which included various combinations of Digitalis, diuretics (Furosemide and/or Spironolactone), an Angiotensin Converting Enzyme Inhibitor (Enalapril), a beta blocker (Carvedilol); with or without addition of a Potassium Supplement and Acetylsalicylic Acid (Aspirin). After normalization of LV dysfunction, following institution of HAART, thirteen patients were completely weaned off antifailure therapy and the remainder were in the process of being weaned off.

Fourteen out of the 16 patients in the HAART naïve group were commenced on Digoxin, Furosemide and Potassium supplements. Two of the 14 patients were also on aspirin treatment. All of the seven patients that were followed up remained on antifailure medication at the last follow up.

### 5.4 Changes in the CD4 T-cell sub-set levels

CD4 T-cell subset and viral load levels from the group 2 patients were not available for documentation since these patients were managed in the pre-HAART era when CD4 counts and viral load testing was not done.

The CD4 percentages rather than absolute counts were used as a measure of immune competence in group 1. The pre-HAART CD4 percentages were only documented in 17 patients and ranged between 1.04% and 29.4% (median, 12%). Following HAART, the CD4 percentage improved significantly and ranged from 9% to 43% (median, 30.5%), (Figure 5.2 and Table 5.2). Patient 9 (Figure 5.2 and Table 5.2) showed a drop in the CD4 percentage from 21% to 14% associated with worsening LV function when the FS decreased from 27% to 10% (Figure 5.1). This deterioration in myocardial function was thought to be due mitochondrial toxicity linked to HAART. Following substitution of Stavudine with Abacavir, there was normalisation of the CD4 count with improved LV function and an increase of FS to 43%**.** Patient 17 (Figure 5.2, Table 5.2) showed a marginal decline in CD4 percentage from 10.2% to 9% coupled with a decline in viral load over a treatment period of 85 months. The LV function improved despite the patient not being fully virally suppressed (Figure 5.1).

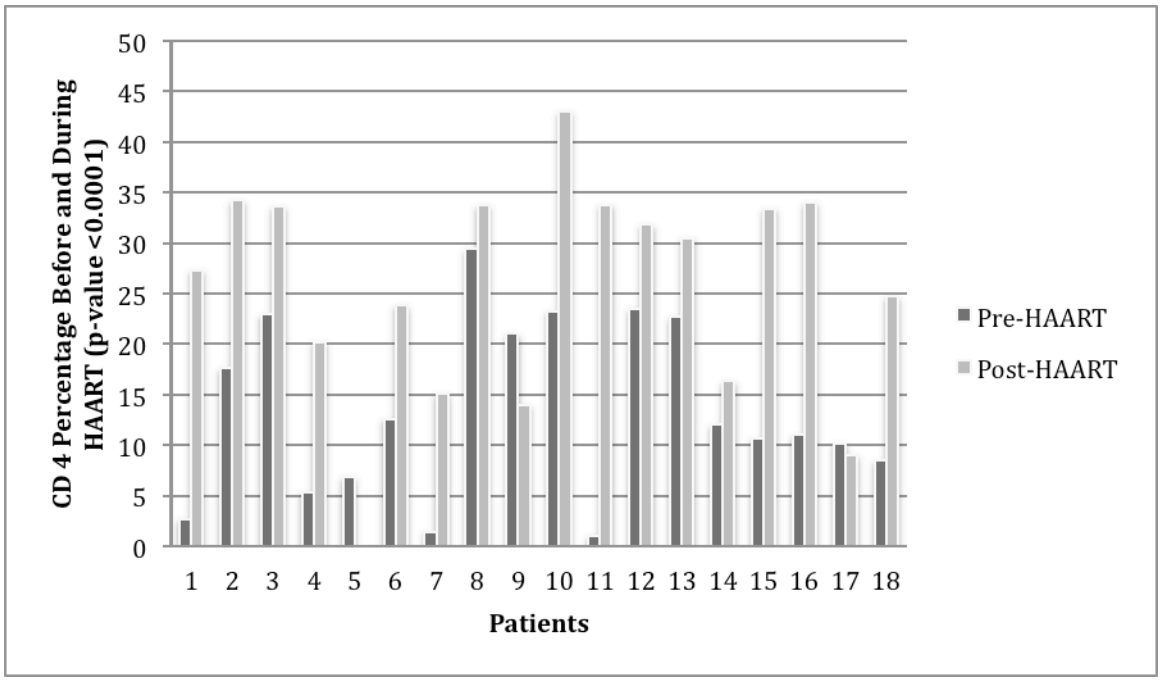


**Figure 5.2. CD4 Percentage Pre-HAART (n=18) and during (post-) HAART (n=17)**

### 5.5 Viral load levels

The pre-HAART viral load of group 1 varied between 6.5 $\log_{10}$ and 3 $\log_{10}$ with a median of 4.4 $\log_{10}$ (Table 5.2). No records of viral load levels were traceable in four patients (patients 8,9,10,13). The overall viral load levels decreased to between 3,5 $\log_{10}$ and undetectable levels (<400 copies/ml) after starting HAART (p <0.0001). The four patients with no documented initial viral load levels were commenced on treatment based on their clinical presentation according to the then National ARV Guidelines (2005).[86]

### 5.6 Trends in nutritional status

#### 5.6.1 Weight-for-age z-scores

The weight-for-age z-scores for group one ranged from -4.75 to 1.61 (median, -1.70) pre-HAART. There was a statistically significant improvement ($p = 0.0083$) of the scores while on HAART to a range of -3.36 to 1.09 (median, -1.32) over a follow up period of 2 weeks to 38 months (median, 21 months) (Table 5.2). Patient 5, (Table 5.2) was omitted from the analysis because no follow up weight-for-age z-scores were documented.

The initial weight-for-age z-scores in group 2 ranged between 1.47 and -3.69 (median, -2.47). There was poor follow up of these patients and the weight-for-age z-scores were available in only five patients.

#### 5.6.2 Height-for-age z-scores

Only 17 patients in group 1 had their height recorded pre-HAART and during HAART (Table 5.2). The initial height-for-age z-scores ranged between -4.6 and -0.64 (median, -2.16). These improved slightly to a range of -4.19 and -0.54 (median, -1.71) over a follow up period of 2 weeks to 38 months (median = 21 months). The difference in height-for-age z-scores pre-HAART and during HAART was statistically insignificant ($p= 0.076$).

The initial height-for-age z-scores were available in 14 out of the 16 patients in group 2 and ranged from -4.92 to 9.39 (median, -1.16). The lack of follow up data in 14 patients did not allow for assessment of a trend in height-for-age z-scores over a period of time.

**5.7 Anti-retroviral Therapy combinations.**

All patients in group one received ARV's according to the National ARV protocol (2005),[86] and were treated for a duration of 1 to 85 months (median, 23 months). The anti-retroviral drug combinations, substitutions and reasons for change are presented in table 5.2.

Patient 3 (table 5.2) was diagnosed with HIV associated dilated cardiomyopathy prior to starting HAART therapy (Stavudine, Lamivudine and Efavirenz). After eleven months on this regimen, the patient had temporary normalisation of myocardial function which was paralleled by viral suppression and an improved CD4 percentage. The patient then developed lactic acidosis and a return of left ventricular dysfunction which was attributed to mitochondrial toxicity due to HAART. Both Stavudine and Efavirenz were replaced by Abacavir and Kaletra which was followed by resolution of the lactic acidosis and improvement in left ventricular function after sixteen months on the new HAART regimen.

Patient 10 (table 5.2) who had normal myocardial function prior to therapy developed left ventricular dysfunction associated with a moderately suppressed CD4 percentage (23.2%), with complete viral suppression (<400 copies/ml) while being treated with Stavudine, Lamivudine and Efavirenz after 6 months of treatment. The myocardial dysfunction was presumed to be due to mitochondrial toxicity secondary to HAART. Stavudine was substituted with Abacavir and this was followed by improvement in LV function after eleven months of the revised treatment regimen.

Patient 17 (Table 5.2) developed myocardial dysfunction while receiving HAART which included a combination of Didanosine, Zidovudine and Efavirenz. This patient was the only patient who was started on HAART before the national roll-out of ARV's and was a referral from the PHRU with heart failure whilst on antiretroviral therapy. A myocardial biopsy was undertaken and histological examination showed features of mitochondrial toxicity. Resolution of myocardial dysfunction in this patient followed a change in therapy to Lamivudine, Abacavir and Kaletra.

One patient (patient 12) had normalization of LV dysfunction within one month of therapy. The rapid recovery of myocardial function suggests the possible diagnosis of a myocarditis rather than a dilated cardiomyopathy in this patient.

**5.8 Outcome**

Outcome was recorded as death, lost to follow up and possibly dead, alive with LV dysfunction, or alive with normal or recovering LV function.

Seventeen patients in group one were alive and one patient died from possible rupture of a Wilm's tumour at termination of follow up. Fifteen of the patients who survived had normal LV function (FS > 25%) after a treatment duration of 11 months to 85 months (median, 23 months). Only two patients had subnormal LV function (Patients 6 and 9, Figure 5.1, Table 5.2) after the follow up period. Patient 6 was initially treated with Stavudine, Lamivudine and Efavirenz, but was not compliant with medications and developed resistance to Efavirenz which was substituted with Kaletra, and Abacavir was added to the regimen. This change was followed by recovery of LV function from an initial low FS of 7% to 19% at the last follow up. Patient 9 (Figure 5.1, Table 5.2) had a FS of 27% prior to HAART. The FS

deteriorated to 10% after 24 months of HAART. HAART-induced mitochondrial toxicity was suspected, following which a recommendation was made to change to a less cardiac toxic regimen. Stavudine was then substituted with Abacavir with good recovery of LV function to a normal FS of 43%.

Nine patients were immune competent with CD4 percentages of >25% and completely suppressed viral loads (<400 copies/ml) associated with normalization of LV function at the end of the follow up period. Patients 14 and 18 (Table 5.2) had moderate CD4 percentage improvement with complete viral load suppression, and normal FS by the end of the study follow up. Three patients (patients 4, 6 and 7 in Table 5.2) had moderate improvement in CD4 percentage and incomplete but substantial viral suppression associated with some recovery of function in patient 6 and complete normalisation of LV function in patients 4 and 7. Patient 5 showed recovery of ventricular function but the immune status was not recorded at follow up (Figure 5.1, Table 5.2). Patient seventeen (Table 5.2) had a marginal drop in the CD4 percentage from 10.2% to 9% even though there was viral load suppression (from 4.2 $\log_{10}$ to 3.5 $\log_{10}$) which was associated with normalisation of left ventricular function. Patient nine (Table 5.2), showed a decrease in CD4 percentage from 21% to 14%. However, the viral load remained low (< 400 copies/ml) and there was deterioration of left ventricular function. Mitochondrial toxicity was suspected in this patient and Stavudine was then substituted with Abacavir with LV function recovery to a normal FS of 43%.

Patient 12 (Figure 5.1, Table 5.2) showed normalization of CD4 percentage and substantial viral load suppression (from 4.1 $\log_{10}$ to <400 copies/ml). These changes were paralleled by normalization of LV function. However, the patient demised from suspected ruptured

Wilm's tumour. Overall, recovery of LV function was paralleled by improved immune status in the majority of patients.

The outcome of the patients in Group 2 was very poor. Half of the patients from this group demised, whilst the other half was lost to follow up and as such presumed to have died. The cause of death in 7 patients was taken from patients' in-hospital charts and death certificates. Three patients had congestive cardiac failure reported as the cause of death, although there was other comorbidity mentioned. The remaining 4 patients were reported to have died from other illnesses associated with World Health Organisation Clinical Stage Four of HIV disease[90] or Centre for Disease Control HIV clinical category C disease.[91] One patient was reported to have died suddenly at home soon after discharge.

## Chapter 6. Discussion:

### 6.1 Myocardial Function, HIV Infection and HAART

This study confirms the findings of other investigators[11,13-16,55] that show a statistically significant recovery of myocardial function following institution of HAART in both paediatric and adult patients. The very first report of a paediatric patient who had resolution of dilated cardiomyopathy following HAART treatment was reported in 2003 by Diogenes, et al.[14] In one study that reviewed 1042 adult patients that were HIV positive revealed that 31.3% developed cardiac complications. Nine percent had myocardial dysfunction before introduction of HAART.[11] This figure dropped to 2.2% following HAART. Herdy, et al[13] prospectively studied 47 paediatric patients with HIV and cardiovascular complications. Ten percent of these patients had LV dysfunction. The patients were initially treated with

monotherapy using Zidovudine, which was associated with high mortality and failure of resolution of LV dysfunction. Introduction of HAART in the survivors was associated with normalisation of LV function. In 2004, Plebani et al, reported five children who had dilated cardiomyopathy before commencement of antiretroviral therapy. [15] Two patients received dual therapy (Zidovudine and Lamivudine) without improvement in LV dysfunction, before being changed to HAART. All five patients subsequently recovered their ventricular function with normalisation of fractional shortening following introduction of HAART over a period of six months. Another patient which was part of a published paediatric case series,[16] was commenced on Zidovudine monotherapy at nine weeks of age following a diagnosis of *pneumocystis carini* (now *jiroveci*) pneumonia, developed a dilated cardiomyopathy at nine months of age. The patient was changed to Didanosine without improvement in LV dysfunction. At 3.5 years of age, this patient was commenced on HAART and after one year of treatment was found to have a normal FS of 33%. An adult based study,[55] has shown that introduction of HAART, not only reversed myocardial dysfunction, but led to a 30% reduction in HIV-associated LV dysfunction incidence. Two patients in our study case series developed left ventricular dysfunction while on HAART. A myocardial biopsy performed in one patient showed histological features of mitochondrial toxicity which is a well known complication of HAART, in particular of Nucleoside Reverse Transcriptase Inhibitors such as Zalcitabine, Didanosine, Stavudine and Zidovudine.[57,58] The histological changes and myocardial dysfunction associated with mitochondrial toxicity have been shown to resolve over time after substitution with less cardiotoxic drugs. [60,61,85]

### 6.2 Cardiac Failure Medication

All patients with left ventricular dysfunction were commenced on antifailure medication which consisted of digitalis, diuretics, angiotensin converting enzyme inhibitors, potassium supplementation and from the mid 2000s, Carvedilol which is a B-blocker, was added to the antifailure treatment regime in patients with severe LV dysfunction. In this study, only six of the 18 group one patients received Carvedilol. Aspirin was also added as an anti-thrombotic agent in patients with very poor LV function who are at risk of forming intracardiac thrombi. Antifailure medication is usually prescribed for symptomatic relief in patients with myopathic hearts, which may delay progression of the disease and prolong survival of the patients without necessarily leading to normalisation of LV function. It is unlikely, therefore, that the improvement of myocardial function in the Group one patients was due to the addition of antifailure treatment alone. The group two patients were shown to have a less favourable outcome despite being on antifailure treatment. Although these patients were not provided with the newer additional therapies such as Carvedilol, they were clearly further disadvantaged by not having access to ARVs as well. The combined absence of these medications in their treatment regime clearly affected their cardiac prognosis.

### 6.3 Immunological Status and Myocardial Function in HIV Infection

It is apparent that immunological recovery parallels the improvement in left ventricular function, which is evidenced by an increase in CD4 percentage and viral load suppression following introduction of HAART. The most likely explanation is that HIV induced immuno-deficiency exposes patients to devastating opportunistic infections[86,92] which may cause

myocarditis and subsequent dilated cardiomyopathy with ventricular dysfunction.[2,36, 45-47] In addition, HIV itself has been demonstrated within cardiomyocytes and may directly affect the myocyte cytoskeleton causing cleavage of dystrophin resulting in left ventricular dysfunction and dilated cardiomyopathy.[32-35,40]

Commencement of HAART is coupled with recovery of the immune system and the concomitant elimination of infections that cause myocardial dysfunction.[11,55] Opportunistic cardiovascular infections in HIV infection themselves are associated with severely depressed immune function (low CD4 percentage and high viral load) which leads to a vicious cycle of persistent infection.[2-8] One of the study patients (patient 6, figure 5.1, Tables 5.2 and 5.3), who was not compliant with treatment demonstrated no recovery of LV function while on HAART due to the development of drug resistance. There was a remarkable improvement in LV function and immune status after a change in the treatment regime and improved compliance. This observation suggests that improperly treated HIV infection with failure of viral suppression allows continued viral replication in the myocardial tissue resulting in myocardial dysfunction. Once viral suppression has been induced the viral replication process is halted and results in recovery of myocardial function. One can conclude therefore, that the HIV itself results in myocardial inflammation which causes damage to the myocardial tissue which manifests with ventricular muscle dysfunction.[32-35,40,93]

### 6.4 Nutritional Status and Left Ventricular Function in HIV Infection

The majority of the patients in both groups had initial weight-for-age (29 out of 34 patients) and height-for-age (26 out of 34 patients) z-scores below the median for age. This was not

an unexpected finding since HIV infection has been associated with failure to thrive and has been described previously as "a wasting syndrome".[92] In addition, myocardial dysfunction in patients with advanced HIV infection has been documented to be associated with an increase in morbidity and mortality.[36] The institution of HAART in the study patients was coupled with improved LV function which paralleled the recovery of their nutritional status. This association has been documented elsewhere in the literature.[55,56]

### 6.5 Outcome

Not only did the majority of the patients in group one survive but they demonstrated improvement of cardiac function, immune status and nutrition with the addition of HAART. The Group 2 patients, either died or were lost to follow up and were presumed to have died. This underscores the importance of early commencement of HAART in patients with left ventricular dysfunction which results in improved patient survival.

## Chapter 7. Study Limitations, Conclusion and Recommendations

### 7.1 Study Limitations

Not every patient with HIV referred to the centres where the study patients were selected from underwent cardiac examinations to exclude myocardial dysfunction. The true impact of HIV associated myocardial dysfunction cannot therefore be assessed. The study undertaken was retrospective with a small cohort of patients some of whom were referred with cardiac symptoms which introduced a selection bias and did not allow for a proper analysis of HIV and its cardiac complications within the wider HIV infected paediatric population.

## 7.2 Conclusion:

HIV infection is a devastating condition that leads to suppression of the immune system and results in weight loss and growth failure. Cardiovascular complications such as LV dysfunction and heart failure appear to be associated with advanced disease once patients have developed immune suppression and failure to thrive. This study has affirmed the important role of HAART which contributes to improved nutritional status and normalisation of myocardial function in patients with HIV associated left ventricular dysfunction. An awareness of the possibility of HAART associated ventricular dysfunction in patients who are non-compliant with medications and also those that may develop mitochondrial toxicity when placed on certain NRTI's needs to be made amongst medical staff manageing HIV positive children on HAART. Reinforcement of compliance and substitution of possible cardiotoxic medications respectively, should become routine management of these complications.

## 7.3 Recommendations

A prospective study of patients with HIV presenting with clinical features of, and referred for echocardiographic assessment for LV dysfunction in the HAART era may offer an excellent opportunity to properly elucidate the underlying causes of LV dysfunction as well as to monitor progress and development of HAART related adverse effects, such as mitochondrial toxicity. The commencement of HAART and review of failing HAART regimens in all patients with LV dysfunction is recommended.

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**Appendix 1**

**The Paediatric HAART Combinations from the National Guidelines (2005)[86]**

**6 months up to 3 years**

**First Line**

Stavudine (d4T)

Lamivudine (3TC

Ritonavir/Lopinavir (Kaletra)

**Second-line**

Zidovudine (AZT)

Didanosine (ddI)

Nevirapine or Efavirenz

**Over 3 years and >10 kg**

**First Line**

Stavudine (d4T)

Lamivudine (3TC)

Efavirenz (EFV)

**Second Line**

Zidovudine (AZT)

Didanosine (ddI)

Ritonavir/Lopinavir (Kaletra)

## Appendix 2 : DATA COLLECTION SHEET

**Patient Study Number:….. DOB:…… Sex… Age (months) at HAART Rx:…..**

**Age at LV dysf Dx….**

**Anthropometry /Percentiles**

| | **Pre-HAART** | | **6/12 on HAART** | | **1yr on HAART** | | **18/12 on HAART** | |
|---|---|---|---|---|---|---|---|---|
| Date: | …… | | …… | | …… | | ……. | |
| | Anthro. | Perc. | Anthro. | Perc. | Anthro. | Perc. | Anthro. | Perc. |
| Weight | …… | | ……. | | ……. | | ……..... | |
| Height | …… | | ……. | | ……. | | ……..... | |
| OFC | …… | | ……. | | ……. | | ……..... | |
| Z-Score | …… | | ……. | | ……. | | ……. | |

**HIV Diagnosis:**

**Date: Test: HIV Elisa PCR**

**CD4 Percentage and Viral Load Levels:**

| | **Pre-HAART:** | **6/12 on HAART** | **1yr on HAART** | **18/12 on HAART** |
|---|---|---|---|---|
| CD4: | …… | …….. | ……. | …….. |
| Viral load: | …… | …….. | ……. | ……..... |
| Date: | …… | …….. | ……. | ……..... |

**Highly Active Anti-Retroviral Therapy**

Date: …….. …….. …….. ……..

| First Regimen | Second Regimen | Third Regimen | Additional Drugs |
|---|---|---|---|
| | | | |
| | | | |
| | | | |

**Date and Reasons for change of Regimen:**

Date:…….. Reasons:……

Date:……. Reasons:……

**ECHOCARDIOGRAPHICAL ASSESSMENT SUMMARY:**

**Initial**

**Date:**

**Follow up**

**Date:**

**Follow up**

**Date:**

**ANTIFAILURE MEDICATION COMBINATIONS:**

**OUTCOME:**