Central University of Technology, Free State

The Influences of CD4 cell count, Viral load and Antiretroviral Treatment on Left Ventricle Ejection Fraction of Adult HIV/AIDS Patients

Sonja Steenkamp

Dissertation submitted in fulfilment of the requirements for the Degree

MAGISTER TECHNOLOGIAE:
CLINICAL TECHNOLOGY: CARDIOLOGY

in the

School of Health Technology
Faculty of Health and Environmental Sciences

at the
Technikon Free State

Studyleaders: Dr M Makotoko (MBChB, MRCP, FCP)
Dr WMJ van den Heever (PhD)

BLOEMFONTEIN
January 2004

Central University of Technology, Free State

Declaration

Declaration with regard to independent work:

I, SONJA STEENKAMP, identity number [redacted] and student number [redacted], do hereby declare that this research project submitted to the Technikon Free State for the Degree MAGISTER TECHNOLOGIAE: CLINICAL TECHNOLOGY: CARDIOLOGY, is my own independent work; and complies with the Code of Academic Integrity, as well as other relevant policies, procedures, rules and regulations of the Technikon Free State; and has not been submitted before to any institution by myself or any other person in fulfilment (or partial fulfilment) of the requirements for the attainment of any qualification.

Steenkamp
SIGNITURE OF STUDENT

12 January 2004
DATE

Acknowledgements

I would like to acknowledge the following people for their input regarding this thesis:

- Mrs Ina Bester, Department of Biostatistics, University of the Free State; for her guidance and help with the statistics.
- Mr Sabbagha, for his linguistic care.
- All the willing patients that put their trust in us. Dr Makotoko and I appreciate their unselfish act of willingness to participate in this research project, which has contributed in the quest for greater understanding of this complex condition.

I would also like to thank the Technikon Free State for the honour of being granted the Innovation Fund scholarship. Without this generous scholarship, my studies would have been impossible.

To my two wonderful supervisors – Dr M Makotoko and Dr WMJ van den Heever – I would like to direct a special word of thanks. I appreciate the dedication, time, effort and teaching they devoted to this thesis and to me. Thank you for your support and encouragement when I really needed it most.

To my husband, Danie, for his patience and understanding and to my mother who brought me to this point in my life and supported me throughout: thank you.

Above all, to my Heavenly Father, who made everything possible. To Him who gave me the strength to see it through, I am most grateful.

Sonja

Table of Contents

List of Tables

List of Figures

List of Abbreviations

AIDS:	Acquired immunodeficiency syndrome.
ARB:	Antiretrovirale behandeling.
ART:	Antiretroviral therapy.
BDNA:	Branched DNA.
CW	Continuous Wave Doppler.
DNA:	Deoxyribonucleic acid.
DNTPs:	Deoxynucleoside triphosphates.
HAART:	Highly active antiretroviral therapy.
HIV:	Human immunodeficiency virus.
ISH:	In situ hybridization.
Log:	Logarhythm.
LVEF:	Left ventricle ejection fraction.
LVUF:	Linker ventrikulêre uitwerp fraksie.
MIV:	Menslike immuniteitgebrek virus.
MTCT:	Mother to child transmission.
NASBA:	Nucleic acid sequence based amplification.
NNRTI:	Non-nucleoside reverse transcriptase inhibitors.
NRTI:	Nucleoside reverse transcriptase inhibitors.
PCR:	Polymerase chain reaction.
PI:	Protease inhibitor.
RNA:	Ribonucleic acid.

RTI: Reverse transcriptase inhibitors.

RT-PCR: Reverse transcriptase polymerase chain reaction.

VIGS: Verworwe immuniteitgebrek-sindroom.

WHO: World Health Organisation.

List of Definitions of Key Terms

Left Ventricle Ejection Fraction (LVEF): The volume of blood ejected from the left ventricle during each systole can be expressed as a percentage of the end-diastolic volume. This gives the ejection fraction. The normal LVEF value is between 52% and 75% (Anderson *et al.*, 1993).

Echocardiography: This produces cardiac images by reflecting pulses of ultrasound from the structures of the heart and then recording the reflected sound signals (Anderson *et al.*, 1993).

CD4: T-helper cell (Miller, 2000b).

CD4 Cell Count: A measure of the cumulative damage caused to the immune system by infection with HIV. It is measured as cells/ml (Allen *et al*, 2000). The normal value range for the CD4 cell count is between 510 – 1310 cells/ml (University of the Free State, 1997/8).

Viral Load: The level of virus in plasma (Allen *et al,* 2000). By six months after infection, viraemia stabilizes at a constant level, or "set point". The quantity of viral RNA measured in the blood after this set point has been achieved, is known as the "viral load" (Miller, 2000b). In patients without HIV/AIDS infection

there will be no HIV RNA copies in the blood. Therefore the normal value for HIV viral load will be 0 RNA copies/ml.

HAART: Highly active antiretroviral therapy consists of a multi-drug combination, usually three but can also be four, using drugs that inhibit different steps in the HIV replication cycle to achieve the greatest viral suppression. This can be two NRTIs plus one PI or NNRTI, or two PIs or various other combinations (Makotoko, 2003).

Summary

This research project compared the influence of the CD4 cell count, the viral load and antiretroviral therapy (ART) on the left ventricle ejection fraction (LVEF). The purpose was to see what the relationship between the variables was in an attempt to find a point in the course of the HIV/AIDS disease where it is necessary to do an echocardiogram on these patients to evaluate the LVEF. All the data of the variables were included against the inclusion and exclusion criteria. An echocardiogram was done on all those HIV/AIDS diagnosed patients who gave their consent to evaluate the LVEF. The patients then went for their usual blood tests and ART was given if it was indicated. The findings show that there was a direct proportional relationship between the LVEF and the CD4 cell count, and an indirect proportional relationship between the viral load and the LVEF. The prevalence of a below normal LVEF was mostly found in patients whose CD4 cell count was below or equal to 100 cells/ml and/or the viral load above or equal to 100 000 RNA copies/ml. The majority of patients whose LVEF was below normal and whose viral load was equal to or below 100 000 RNA copies/ml were on antiretroviral therapy. When the viral load exceeded the cut-off point of 100 000 RNA copies/ml, the patients who were not on ART with a below

normal LVEF exceeded those who were on ART. The majority of patients with a low CD4 cell count whose LVEF was below normal were not on antiretroviral therapy. It was also found that the ART had a direct positive effect on the CD4 cell count and the viral load, and indirectly on the LVEF. Therefore, because of the relationship between the CD4 cell count and the LVEF and the viral load and the LVEF, ART had an indirect effect on the LVEF via the CD4 cell count and/or the viral load. This means that if there was an increase in the CD4 cell count due to the ART, then one could expect to see a rise in the LVEF because of the direct proportional relationship between the CD4 cell count and the LVEF. If the ART led to a decrease in the viral load, then one could expect that the LVEF would increase due to an indirect proportional relationship between the viral load and the LVEF. Successful antiretroviral therapy had a positive effect on the CD4 cell count and the viral load, and an indirect positive effect on the LVEF (via the CD4 cell count and/or the viral load).

To conclude, the treating physician of an adult HIV/AIDS patient should consider an echocardiogram on all these patients when the CD4 cell count falls below 100 copies/ml, and/or when the viral load exceeds 100 000 RNA copies/ml in order to identify those patients who have left ventricular dysfunction and who might therefore benefit from treatment with appropriate medication.

Opsomming

Hierdie navorsingsprojek het die invloed van die CD4 seltelling, die viruslading en die antiretrovirale behandeling (ARB) op die linker ventrikulêre uitwerpfraksie (LVUF) bestudeer. Die doel was om te sien wat die verwantskap was tussen hierdie veranderlikes in 'n poging om 'n punt vas te stel in die verloop van die MIV/VIGS siekte waar dit belangrik is om 'n eggokardiogram te doen om die LVUF te bepaal. Alle data en veranderlikes was ingesluit teen die insluitings- en uitsluitingskriterias. 'n Eggokardiogram is gedoen op alle MIV/VIGS pasiënte wat toestemming gegee het, om die LVUF te evalueer. Die pasiënte het daarna vir hulle gereelde bloedtoetse gegaan en ARB is, indien nodig, vir hulle voorgeskryf. Die bevindinge wys dat daar 'n direkte eweredige verwantskap is tussen die CD4 seltelling en die LVUF en dat daar 'n indirekte eweredige verwantskap is tussen die viruslading en die LVUF. Die voorkoms van die LVUF om onder normaal te wees, het die meeste voorgekom by pasiënte wie se CD4 seltelling onder of gelyk aan 100 selle/ml was en/of wie se viruslading bo of gelyk aan 100 000 RNA kopië/ml was. Uit alle pasiënte wie se LVUF onder normaal was en virusladingtoetse gehad het, was die meeste op ARB tot en met die afsnypunt van ≤100 000 RNA kopië/ml. Wanneer die viruslading die afsnypunt van 100 000 RNA kopië/ml oorskry het, was die pasiënte wat op geen ARB was meer as

die wat op ARB was. Uit alle pasiënte wat 'n CD4 seltelling en 'n LVUF van onder normaal gehad het, was die meeste op geen ARB nie. Daar is ook gevind dat die ARB 'n positiewe uitwerking op die CD4 seltelling en/of die viruslading gehad het, maar nie 'n direkte invloed op die LVUF nie. Daarom, as gevolg van die verhouding tussen die CD4 seltelling en die LVUF en die viruslading en die LVUF, het die ARB 'n indirekte effek op die LVUF via die CD4 seltelling en/of die viruslading. Dit beteken dat 'n toename in die CD4 seltelling as gevolg van die ARB, heel moontlik 'n toename in die LVUF tot gevolg het, as gevolg van hulle direkte eweredige vewantskap. Indien die ARB tot 'n afname in die viruslading lei, kan 'n toename in die LVUF verwag word, as gevolg van die indirekte eweredige verwantskap tussen hulle. Die suksesvolle antiretrovirale behandeling het 'n positiewe effek op die CD4 seltelling en op die viruslading en 'n indirekte positiewe effek op die LVUF (via die CD4 seltelling en/of die viruslading).

Die slotsom is dus dat alle behandelende geneeshere van MIV/VIGS pasiënte 'n eggokardiogram goed moet oorweeg, sodra die virus lading bo 100 000 RNA kopië/ml is en/of die CD4 seltelling onder 100 selle/ml is, sodat die pasiënte met linkerventrikulêre wanfunksie geïdentifiseer kan word en hulle die nodige behandeling daarvoor kan ontvang.

Chapter 1 Introduction

South Africa had an estimated 5 million people – children and adults – living with HIV infection and AIDS at the end of 2001 (UNAIDS/WHO, 2002). The researcher recognised many patients diagnosed with HIV infection that presented with left ventricular dysfunction or congestive cardiac failure. There have been publications in the medical literature linking HIV infection and AIDS with left ventricular dysfunction but none from South Africa (Currie *et al.,* 1998; Schlant & Alexander, 1994; Lipshultz *et al.,* 1998; Barbaro *et al.,* 1998b; Hivdent, 1998; Millei *et al.,* 1998; Murphy, 1999; Rerkpattanapipat *et al.,* 2000; Pugliese *et al.,* 2000; Acierno, 1989; Warkentin, 1998; Yunis and Stone, 1998). In South Africa little is known about the influence of HIV/AIDS on the LVEF.

A review by Millei *et al.* (1998) highlighted the fact that there are very few clinical studies on this topic. Current knowledge is based almost exclusively on echocardiography and autopsy studies. Observational or clinical trials based on syndromes of heart failure, tamponade and so forth would be useful. There is also very little information on the impact of antiretroviral therapy on the LVEF. Finally, because cardiac complications are often clinically inapparent or subtle in the initial stages, periodic screening of HIV-positive patients by ECG and echocardiogram is probably indicated. The use of routine electrocardiography and echocardiography for asymptomatic patients with HIV infection is controversial (Millei *et al.*, 1998).

Echocardiographic examination is more informative and sensitive than electrocardiographic monitoring. Indeed, echocardiography appears to be the most appropriate way to detect heart involvement during HIV infection; it allows for early diagnosis and thus provides time to find the most suitable way of treating cardiac abnormalities, even in the early asymptomatic phase of the disease. Prompt recognition and treatment is important because palliative therapy with diuretics and vasodilators can be worthwhile: mild global left ventricular dysfunction appears to be reversible in many patients and a sub-group progresses to symptomatic heart failure without treatment. It appears prudent to perform a careful cardiac examination at the time of diagnosis of HIV to obtain a baseline functional assessment. An echocardiogram should be performed if there are signs of heart disease (Millei *et al.*, 1998; Warkentin, 1998). HIV/AIDS related cardiomyopathy is a diagnostic challenge to which physicians should remain alert (Yunis *et al,* 1998). A need was identified to study further the relationship between the stages of HIV infection as represented by the viral load and CD4 cell count and the left ventricle ejection fraction, as well as the influence that antiretroviral therapy would have on the left ventricle ejection fraction.

Because cardiac complications are often clinically inapparent or subtle in the initial stages, periodic screening of HIV-positive patients by electrocardiogram and echocardiogram is important (Millei *et al.*,

1998). The main focus of this research project was the left ventricle ejection fraction. The influences of the different variables on the left ventricle ejection fraction were measured. The first part of the research was done to see what the relationship was between the CD4 cell count and the left ventricle ejection fraction, and then the relationship between the viral load and the left ventricle ejection fraction. The second part of the research focused on those patients who had a below normal left ventricle ejection fraction (≤52%). Here the researcher wanted to find cut-off points for the CD4 cell count and/or the viral load, where the below normal left ventricle ejection fraction occurred most frequently. As subdivisions of the above mentioned two main parts of the research, the researcher examined the influence of antiretroviral therapy on each.

The main purpose of this research was to find out more about HIV/AIDS, since this devastating disease has a great impact on society and there are still so many unanswered questions. Another purpose was to emphasize the importance of a good, thorough clinical examination of all HIV/AIDS patients by a physician, and the use of an echocardiogram in evaluating the cardiac status of a patient with HIV/AIDS, especially in the advanced stage.

Chapter 2 Literature Review

2.1. Virological and Immunological Events

Following infection by the HI Virus, the virus is transmitted across mucocutaneous membranes. HIV rapidly binds to specialized cells of the macrophage type, known as Langerhan's cells, which transport the virus to regional lymph nodes. This process is probably accomplished within a matter of days. Within the lymphoid tissue, massive replication of HIV occurs, leading to intensive viraemia and widespread dissemination of the virus. During this phase, some 50% of individuals may manifest the clinical signs of the Acute Retroviral Syndrome (Miller, 2000).

Within 6 – 8 weeks, the immune system mounts an aggressive response that includes cytotoxic T-cell activity (causing elevation of the peripheral blood CD8 cell count), antibody evolution (which results in seroconversion in the ELISA and Western Blot test) and cytokine production. In concert, these substantially reduce the level of HIV replication and reduce the level of viraemia. By sixth months after infection, viraemia stabilizes at a constant level or "set point". The quantity of viral RNA measured in the blood after this set point has been achieved, is known as the "viral load". The viral load is one of the two most important prognostic measurements that are regularly monitored in the management of people with HIV infection (Miller, 2000).

During the initial periods of intense viraemia, HIV causes significant depletion of CD4 cells. As viraemia is brought under control by the host's immune system the CD4 count usually increases, but rarely returns to normal levels. Once the set point has been achieved, there is a steady decline in CD4 cells at a rate proportional to the viral load, i.e. high viral loads result in more rapid loss of the CD4 cells and vice versa. On average, without treatment, the CD4 cell count drops at a rate of between 50 – 80 cells/ml/year (Miller, 2000). Over a mean of 8 – 10 years it reaches a low point of <200 cells/ml. A CD4 cell count of <200 cells/ml is indicative of severe immunological depletion; this is the time when the host is most susceptible to the onset of the severe life-threatening opportunistic infections and tumours which define the individual as having AIDS. The CD4 cell count is the second of the two most important monitoring tests used in the management of persons with HIV infection (Miller, 2000).

Complications may occur as a direct result of HIV itself, or as a consequence of the immunosuppression induced by the virus. Illnesses related to HIV itself may occur at any CD4 cell count. Once the CD4 cell count declines below 200 cells/ml the complications regarded as "AIDS-defining" are most prone to occur (Miller, 2000).

The course of HIV infection without treatment averages about 10 years from the time of infection to the onset of AIDS-defining

diseases. In some instances, however, people may lose CD4 cells at an accelerated rate, achieving levels of <200 cells/ml within 2 – 4 years; such individuals are termed "rapid progressors". Conversely, there are people in whom the CD4 count remains above 500 cells/ml for over 8 years in the absence of treatment; these people are termed "non-progressors". These variations in the course of HIV infection may be due to several factors; pre-eminent amongst these is the viral load (Miller, 2000).

There is a strong correlation between the viral load, as measured by HIV RNA levels, and rates of clinical progression. In general, individuals with a viral load of <5 000 RNA copies/ml tend to survive beyond 10 years without antiretroviral therapy and have an associated CD4 cell loss of <50 cells/year (Miller, 2000). Between 5 – 7% of HIV infected individuals fall into this category. Over 80% of HIV-infected persons have viral loads between 20 000 – 80 000 RNA copies/ml and – without treatment – remain well for 8 – 10 years after infection. They experience an average CD4 cell loss of 50 – 80 cells/year and are termed "average progressors". Up to 10% of HIV-positive persons have viral loads of >100 000 RNA copies/ml; they tend to progress to AIDS within 2 – 4 years and are termed "rapid progressors"; the annual decline in CD4 cells exceeds 80 cells/year in this group (Miller, 2000)

Reducing the viral load to those levels seen in non-progressors is the general goal of HIV therapy. This is associated with a substantial alteration in the natural history of HIV infection and results in the majority of individuals remaining clinically well for prolonged periods of time (Miller, 2000).

2.2. Cardiomyopathy, Antiretroviral Therapy and HIV/AIDS.

There is an increased prevalence of cardiomyopathies in patients with the acquired immunodeficiency syndrome (AIDS). Dilated cardiomyopathy is strongly associated with a CD4 cell count of <100 cells/ml, in contrast with the other forms of cardiac dysfunction (Currie *et al.*, 1998). Echocardiographic evidence of left ventricular dysfunction is more common in patients who are the furthest along in the course of HIV disease. Individual reports of one to five cases of patients with either dilated left ventricle, hypokinetic left ventricle or both have been reported frequently enough to require explanation. Furthermore, the frequent occurrence of cardiomyopathy in children who have HIV/AIDS, further suggests a relationship between HIV disease and cardiomyopathy (Schlant & Alexander, 1994). Lipshultz *et al.* (1998) reported that the degree of depression of LVEF correlates with the extent of immune dysfunction at base line but not in the long term, suggesting that the CD4 cell count may not be a useful surrogate marker of HIV-associated left ventricular dysfunction.

HIV infection is increasingly recognised as an important cause of dilated cardiomyopathy. During a study compiled by Barbaro *et al.* in 1998 with a mean follow-up period of 60±5.3 months, an echocardiographic diagnosis of dilated cardiomyopathy was made in

76 patients (8%) with a mean annual incidence rate of 15.9 cases per 1 000 patients (Barbaro, 1998b). According to a study published in the Hivdent in 1998, clinical cardiomyopahty was seen in 1 to 4% of AIDS patients, with patients frequently asymptomatic (Hivdent, 1998). Ince (1999) also found that 8% of the cohort study they compiled had dilated cardiomyopathy and that the greatest incidence of dilated cardiomyopathy was in those with depressed CD4 cell counts; in particularly in those with CD4 cell counts <300 cells/ml. In a review written by Millei *et al.* (1998) they described a few different prevalences. During one of the studies (Anderson and Virmani, 1990) that Millei *et al.* (1998) reviewed, cardiovascular disease occurred in approximately 6.5 – 6.8% of HIV infected persons. When cardiac dysfunction does develop, the signs and symptoms are often misinterpreted as being the result of non-cardiac causes (pulmonary infection or respiratory failure) that can mimic heart failure (Millei *et al.*, 1998). In another study (Hakim *et al.,* 1996) reviewed by Millei *et al.* (1998), which was a prospective survey of 157 acutely ill HIV-positive patients in a hospital in Zimbabwe, they found that the most common echocardiographic abnormalities were left ventricular dysfunction with a prevalence of 22% (Millei *et al.*, 1998). Some other studies (De Castro *et al.,* 1992) reviewed by Millei *et al.* (1998) found that the echocardiographic findings of dilated cardiomyopathy have been reported in 30 – 40% of patients with AIDS and in another study (Longo Mbenza *et al.,* 1995) it was found in 16.9% of patients.

Dilated cardiomyopathy occurs late in the course of HIV infection and is usually associated with a significantly reduced CD4 cell count and with symptoms and signs that are attributed to other disease processes. Millei *et al.* (1998) concluded that left ventricular dysfunction appears to be increased in patients with low CD4 cell counts, although other clinical markers of susceptibility have not yet been well defined. In this connection, echocardiographic examination seems advisable every 6 months. Murphy (1999) found, in a study conducted in Italy in 1997, that 25% of cardiac diseases in HIV patients showed dilated cardiomyopathy and reversible hypokinesia. Another study in Italy (Lipshultz, 1998) published in October 1998 showed that 8% of HIV/AIDS patients developed dilated cardiomyopathy especially when the CD4 cell count was <400 cells/ml (Murphy, 1999). Rerkpattanapipat *et al.* (2000) found that the prevalence of dilated cardiomyopathy ranges from 10 – 30% in echocardiogram and autopsy studies. Patients with severe symptomatic heart failure usually had a low CD4 cell count. Rerkpattanapipat *et al.* (2000) emphasised that Coudray and colleagues demonstrated in 1995 that left ventricular impairment could occur in the early stages of HIV infection. Although the prevalence of dilated cardiomyopathy is higher in HIV infected patients with low CD4 cell counts, there was no association between the progression of left ventricular dysfunction and the rate of CD4 cell count decline (Rerkpattanapipat *et al.,* 2000). Putting together the

prevalence rates of dilated cardiomyopathy among HIV infected patients from the different authors (Currie *et al.,* 1998; Schlant & Alexander, 1994; Lipshultz, 1998; Barbaro *et al.,* 1998b; Hivdent, 1998; Millei *et al.,* 1998; Murphy, 1999; Rerkpattanapipat *et al.,* 2000; Pugliese *et al.,* 2000; Acierno, 1989; Warkentin, 1998; Yunis and Stone, 1998), it appears that the rates range from as little as 1% to as much as 40% in some studies. Most of the studies done agreed that the prevalence of cardiomyopathy in HIV/AIDS patients was particularly high in those with a low CD4 cell count, mostly with a CD4 cell count of less than 300 cells/ml.

Clinical evidence of cardiac disease is usually overshadowed by manifestations in other organs, primarily the brain and lungs. As a consequence, the number of patients with AIDS with cardiac involvement at autopsy greatly exceeds the number with significant cardiac disease during life. Cardiac abnormalities are found at autopsy in two-thirds of patients with AIDS (Millei *et al.*, 1998). The heart is often the unrecognized target of AIDS associated lesions even in the initial phase of the AIDS outbreak (1981 – 1989) (Millei *et al.*, 1998). Although cardiac disease can occur at any stage of HIV infection, cardiac morbidity and mortality are more common in advanced stages (Millei *et al.*, 1998). A study conducted in Italy in August 1998 showed that out of 440 AIDS autopsies, 82 had cardiac involvement, of which 12 had dilated cardiomyopathy (Murphy, 1999). Most of the

authors agreed that the number of cardiomyopathies found at autopsies were more than those found during life. The most common reason for this finding is that the medical field is still very unaware of the fact that cardiac dysfunction occurs in HIV/AIDS patients.

Treatment with the antiretroviral drug zidovudine has been linked to the development of dilated cardiomyopathy (Barbaro *et al.*, 1998b; Ince, 1999), but Barbaro *et al.,* 1998b) felt in the end that the difference observed in the incidence of dilated cardiomyopathy among the risk groups was influenced more by the extent of immunodeficiency than by the type of antiretroviral therapy (Barbaro, 1998b). Rerkpattanapipat *et al.* (2000) also found cardiac dysfunction in adults and children treated with zidovudine (Rerkpattanapipat *et al.*, 2000). In contrast with the findings by Ince *et al.* (1999), Barbaro (1998b) and Rerkpattanapipat *et al.* (2000), a study done by Lipshultz (1998) indicated that zidovudine neither worsened nor ameliorated progressive cardiac changes in HIV infected patients (Lipshultz, 1998). Highly active antiretroviral therapy (henceforth referred to as HAART) does dramatically decrease the incidence of cardiac involvement in patients with HIV infection, in comparison with patients who were only treated with one nucleoside reverse transcriptse inhibitor (henceforth referred to as NRTI). In patients treated with NRTI, 8.1% had dilated cardiomyopathy compared to 1.8% of patients treated with HAART (Pugliese *et al.,* 2000).

To conclude, some of the medical studies (Barbaro, 1998b; Rerkpattanapipat *et al.*, 2000; Ince *et al.,* 1999; Lipshultz, 1998, Pugliese *et al.,* 2000) found that zidovudine was associated with a high incidence of cardiomyopathies in HIV/AIDS patients, while others disagree with this statement, but as Barbaro (1998b) stated, in the end the difference observed in the incidence of dilated cardiomyopathy among the risk groups was influenced more by the extent of immunodeficiency than by the type of antiretroviral therapy used.

Barbaro *et al.* (1998b) concluded that dilated cardiomyopathy may be related either to a direct action of HIV on the myocardial tissue or to an autoimmune process induced by HIV.

The HIV-1 genome has been demonstrated within myocytes at autopsy and biopsy tissue from patients with congestive cardiomyopathy, suggesting a direct cytopathic effect by the virus. Nevertheless, the pathogenesis of the heart muscle disease in AIDS is still unclear (Barbaro, 1999a).

The clinical manifestations of AIDS-associated dilated cardiomyopathy are similar to those of dilated cardiomyopathy of any cause. The echocardiogram is most useful in detecting global hypokinesia with decreased ejection fraction and dilation of the various cardiac

chambers, with abnormal left ventricular end-diastolic and end-systolic dimensions (Acierno, 1989).

Treatment for dilated cardiomyopathy is the same whether or not AIDS is involved (Warkentin, 1998). HIV related cardiomyopathy and associated congestive heart failure might respond to standard management, including inotropic agents, diuretics, vasodilators and angiotensin converting enzyme inhibitors (Yunis *et al.*, 1998).

2.3. Antiretroviral Therapy.

2.3.1. Mode of Action of Antiretroviral Agents

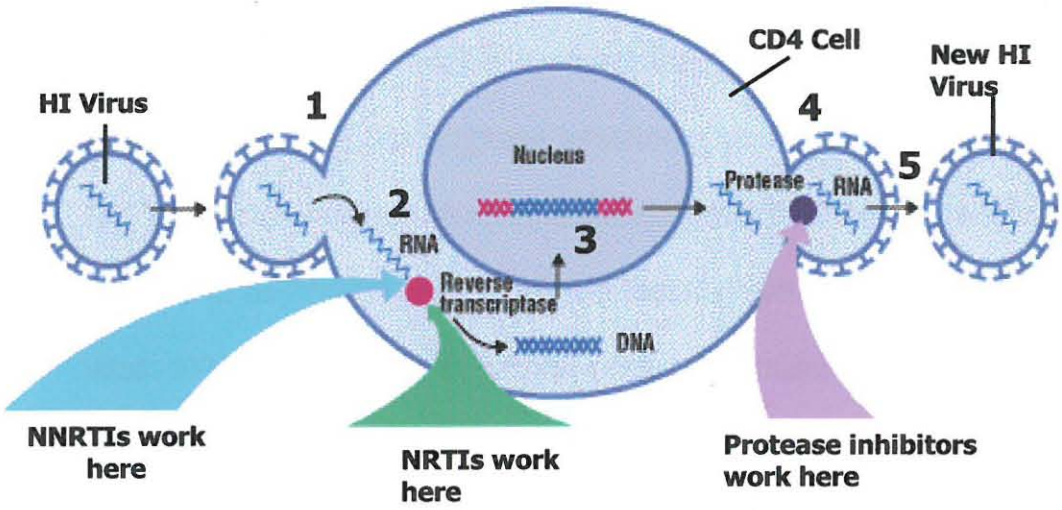

Figure 2.1: *HIV replication and sites of work of antiretroviral drugs.* (Van Dyk, 2001).

Stages of HIV replication (see figure 2.1):

1. HIV enters CD4 cell.
2. HIV is a retrovirus, meaning that its genetic information is stored on a single stranded RNA instead of double stranded DNA found in most organisms. To replicate, HIV uses an enzyme known as reverse transcriptase to convert its RNA to DNA.
3. HIV DNA enters the nucleus of the CD4 cell and inserts itself into the cell's DNA. HIV DNA then instructs the cell to make many copies of the original virus.
4. The proteins required for viral assembly are translated.

5. New virus particles form and leave, ready to infect other CD4 cells (Van Dyk, 2001).

Drugs currently available attempt to block viral replication by inhibiting either reverse transcriptase or the HIV protease enzymes. Those that inhibit reverse transcriptase fall into two classes: Nucleoside reverse transcriptase inhibitors (henceforth referred to as NRTI) and non-nucleoside reverse transcriptase inhibitors (henceforth referred to as NNRTI). These drugs forestall genetic integration of the virus. NRTIs resemble the natural nucleoside building blocks of HIV DNA so that when the reverse transcriptase tries to add the drug to a developing strand of HIV DNA, it cannot be completed (Anderson *et al.*, 2000). The resulting DNA is incomplete and cannot create a new virus (WHO, 2003). NRTIs need to be activated first by phosphorylation (Anderson *et al.*, 2000).

NNRTI act in stopping HIV production by binding directly onto reverse transcriptase (non-competitively) and preventing the conversion of RNA to DNA (WHO, 2003).

Protease inhibitors (henceforth referred to as PI) act at a later stage and interfere with a viral enzyme, HIV protease, which cleaves viral polyproteins into functional end products. This prevents the formation

of mature infectious virus and results in the release of immature non-infectious viral particles (Anderson *et al.,* 1998; WHO, 2003).

Hydroxyurea is indicated in the treatment of certain malignancies and in sickle cell anemia. It has been used investigationally for the treatment of HIV. Hydroxyurea does not have direct antiretroviral activity; rather, it inhibits the cellular enzyme ribonucleotide reductase, resulting in reduced intracellular levels of deoxynucleoside triphosphates (henceforth referred to as dNTPs) that are necessary for DNA synthesis. Depletion of the dNTP pool results in arrest of the cell cycle in the G1 phase prior to DNA synthesis; in an HIV-infected cell, incomplete reverse transcription of the viral genome also results from depletion of the dNTP pool. Hydroxyurea also induces the activity of cellular kinases that phosphorylate nucleoside analogue reverse transcriptase inhibitors, potentially further enhancing their antiretroviral activity (Fauci *et al.*, 1998). Currently, hydroxyurea is no longer in use in the treatment of HIV/AIDS.

2.3.2. Classification of Antiretroviral Therapy

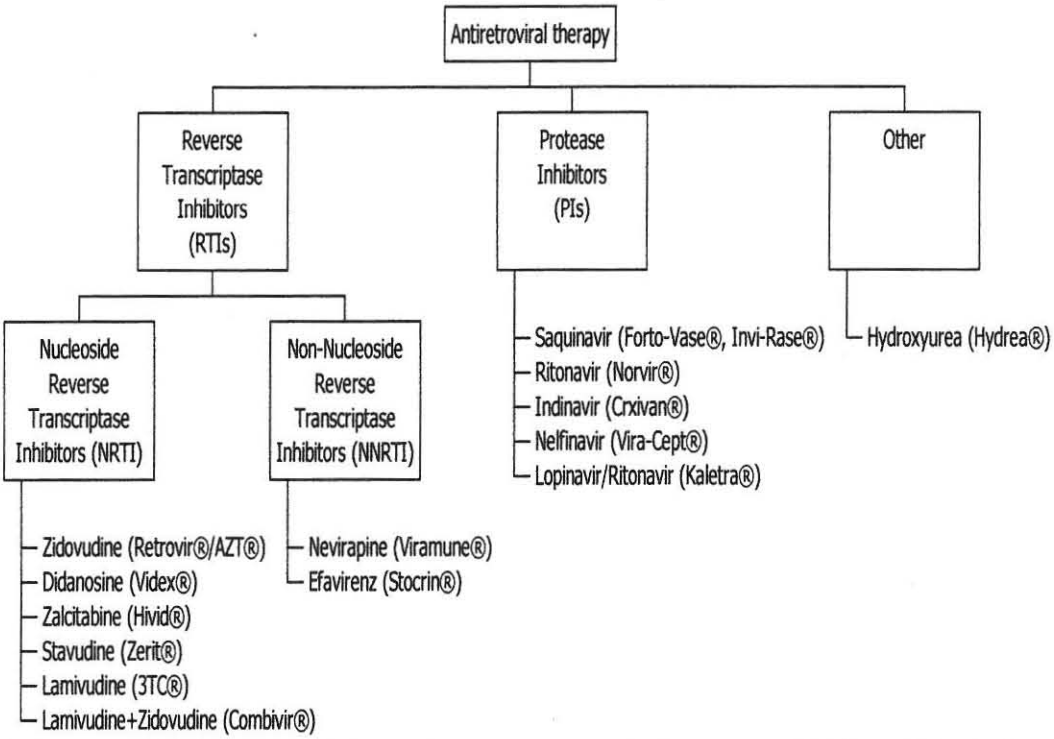

Figure 2.2: Classification of antiretroviral therapy. (Miller, 2002; WHO, 2002)

In accordance with WHO and UNAIDS recommendations, these guidelines endorse the use of NRTIs and NNRTIs as first-line therapy. For initiation of ART therapy two NRTIs and an NNRTI (one drug from Category I, one drug from Category II, and one drug from Category IV) are recommended. If the viral load is <55 000 a third, NRTI (Category III) may be considered as part of a triple NRTI regimen (Miller, 2002; Montaner *et al.*, 1998).

Table 2.1: Guidelines for the use of antiretroviral therapy.

Category I	Category II	Category III	Category IV	Category V
Stavudine Zidovudine	Didanosine Zalcitabine Lamivudine	Abacavir	Nevirapine Efavirenz	Nelfinavir Indinavir Ritonavir Saquinavir Lopinavir/Ritonavir

(Miller, 2002; WHO, 2002)

2.3.3. Goals of Antiretroviral Treatment.

The primary goals of antiretroviral therapy are: (Miller, 2002)

⇨ To prolong life expectancy.

⇨ To improve quality of life.

To achieve these primary goals, one would aim to do the following:

1. Maximal and durable suppression of viral load.
2. Restoration and/or preservation of immunological function.
3. To prevent the development of opportunistic infections and other AIDS related conditions.
4. Reduction of HIV related morbidity and mortality.

Once the decision has been made to initiate ART, the goal is maximum viral suppression for as long as possible (Fauci *et al.*, 1998; Anderson *et al.,* 1998). This is achieved by suppressing viral replication as intensely as possible for as long as possible by using tolerable and sustainable treatment for an indefinite period of time. By doing so, the impact of HIV on the immune system may be minimized and the morbidity and mortality associated with HIV infection can be improved. Effective therapy has been shown to reduce the number of new cells infected by HIV and to impede the ability of the virus to evolve drug resistance (Miller, 2002; Montaner *et al.*, 1998; WHO, 2002).

2.3.4. Initiation of Antiretroviral Therapy.

Treatment should be offered to all patients with the acute HIV syndrome, those within six months of seroconversion and all patients with symptoms ascribed to HIV infection (Fauci *et al.*, 1998).

The latest South African guidelines for the treatment of HIV infection recommend starting antiretroviral therapy at CD4 cell counts of 200 cells/ml or below or for patients with manifestations of opportunistic infections (Southern African HIV Clinicians Society, 2002).

According to the current published international guidelines, the following broad criteria guide the selection of patients for initiation of therapy: (WHO, 2002)

- ⇨ All patients with symptomatic HIV infection regardless of CD4 cell count and viral load levels.
- ⇨ All patients with CD4 cell count below 350 cells/ml.
- ⇨ All patients with a high viral load (>30 000 RNA copies/ml) by RT-PCR.

Current guidelines recommend that treatment be considered for patients in the intermediate range, i.e. plasma viral load between 10 000 – 30 000 RNA copies/ml (RT-PCR) and CD4 cell counts between 350 and 500 cells/ml (WHO, 2002).

Treatment of asymptomatic patients, with CD4 cell counts above 500 cells/ml, is generally deferred as long as the probability of significant immune system damage and of clinical progression of HIV infection remains low (WHO, 2002).

Antiretroviral therapy should be deferred until patients are prepared to commit themselves to long-term treatment and to maintaining good adherence to the therapy. All infected individuals, including those on effective ART, should be viewed as potentially infectious. Adequate counselling about safer sex practices must be provided to encourage prevention of new infections and re-infection (Miller, 2002; WHO, 2002).

Table 2.2: Guidelines for the initiation of antiretroviral therapy.

Symptomatic Patients	**Treatment**
Presence of HIV related symptoms, current or previous HIV associated disease.*	Treatment Recommended
Primary Infection.**	Treatment Recommended
Asymptomatic Patients	**Treatment**
CD4 cell count <200 cells/ml	Treatment Recommended
CD4 cell count 200 – 350 cells/ml	Monitor CD4 cell count and commence treatment if the CD4 annual decline is in excess of the expected 20 – 80 cells/year, or if the CD4 cell count approaches 200.
CD4 cell count >350 cells/ml	Defer Treatment

** These include AIDS defining illnesses (except tuberculosis), unexplained weight loss > 10% of body weight, unexplained diarrhoea > 1 month, oral candidiasis or oral hairy leukoplakia.*

** Primary infection: HAART started early in primary infection leads to viral suppression which appears to maintain HIV specific immunity in a significant proportion of cases who become slow progressors with a low viral load after discontinuing HAART. The duration of treatment is uncertain at the present time.

(Miller, 2002; WHO, 2002).

2.3.5. Prescribed Protocols for Antiretroviral Treatment.

Results of clinical trials to date indicate that the goals may currently be best achieved with a potent PI in combination with two NRTIs. Another option is the combination of saquinavir plus ritonavir combined with one or two NRTIs (Fauci *et al.*, 1998).

Maximally suppressive antiretroviral regimens (HAART) should be used whenever possible in order to obtain the best clinical results and to prevent resistance (Miller, 2002; Montaner *et al.*, 1998; WHO, 2002).

Single drug regimens (monotherapy) should not be used in the treatment of HIV infection; however, it continues to play a very important role in the prevention of mother to child transmission (MTCT) (Miller, 2002; Montaner *et al.*, 1998; WHO, 2002).

Dual drug regimens are moderately effective, but are unlikely to produce long-term durable benefits in most patients. It is not the standard of care, but is considerably better than no therapy and should be considered in patients unable to afford HAART. This should only be applied to patients who have already developed AIDS. In this setting, dual therapy is better than no therapy, otherwise resistance is a major concern if dual nucleoside therapy is prescribed to asymptomatic patients. The efficacy of two drug combinations (dual

therapy) is greater than that of monotherapy, potentially achieving a 1.5 – 1.8 log reduction in viral load. Note that triple combinations are the standard of care (Miller, 2002; Montaner *et al.*, 1998; WHO, 2002).

Triple combination of three synergistic antiretroviral agents remains the standard of care; substantial reductions in medication prices continue to make triple-drug regimens more affordable (Miller, 2002; Montaner *et al.*, 1998; WHO, 2002).

The approaches to antiretroviral therapy and the design of therapeutic regimens have been influenced by the following key findings from studies on the pathogenesis of HIV infection: (WHO, 2002)

- ⇨ Demonstration that a continuous high level of replication of HIV is present from the early stages of infection (at least 10^{10} particles are produced and destroyed each day).
- ⇨ Demonstration that a specific immune response to HIV occurs in HIV infected subjects during "primary" infection followed by a decline after the first months of infection.
- ⇨ Demonstration that the measured plasma viral load is predictive of the sub-sequent risk of disease progression and death.

- ⇨ Proof that combination ART is not only able to consistently suppress HIV replication, but also to induce a significant delay in progression to AIDS; this survival benefit is particularly marked in previously untreated patients.
- ⇨ Elucidation of the molecular, functional and clinical impact of resistance to antiretroviral drugs.

2.3.6. Success versus Failure of Antiretroviral Treatment.

Results of treatment are evaluated primarily with plasma HIV RNA levels; these are expected to show a decrease of one log (10 fold) at 8 weeks and no detectable virus (<50 copies/ml) at 4 to 6 months after initiation of treatment. Failure of treatment (i.e., plasma HIV RNA levels >50 copies/ml) at 4 to 6 months may be ascribed to non-adherence, inadequate potency of drugs or sub optimal levels of antiretroviral agents, resistance and other factors that are poorly understood. Patients whose therapy fails should change to at least two new agents that are not likely to show cross-resistance with drugs given previously (Fauci *et al.*, 1998).

Response to ART is monitored clinically and biologically. The most important biological measurements are the viral load and CD4 cell counts. These measurements correlate with the clinical outcome (WHO, 2002).

2.3.6.1. Criteria for Treatment Success:

- ⇨ A decline in viral load of at least 1 log from pre-treatment levels by 6 – 8 weeks after initiating ART.
- ⇨ A decline in viral load to <50 RNA copies/ml by 24 weeks after commencement of therapy.
- ⇨ A sustained viral load of <50 RNA copies/ml is associated with the most durable virological benefit (Miller, 2002; Montaner *et al.*, 1998).

2.3.6.2. Criteria Indicative of Treatment Failure:

- ⇨ A sustained increase in viral load >50 RNA copies/ml.
- ⇨ A decline in viral load of less than 1 log within 6 – 8 weeks after commencing antiretroviral therapy.
- ⇨ A sustained increase in viral load of >0.6 log from its lowest point or a return to 50% of pre-treatment value.

Inadequate patient adherence to the prescribed regimen remains one of the most common reasons for treatment failure.

Several factors can influence the measurement of HIV viral load. It is strongly recommended that the decision to alter therapy should be based on the results of at least two consecutive viral load measurements performed at least one week apart (Miller, 2002).

2.3.7. Viral Resistance to Antiretroviral Therapy

Viral resistance to antiretroviral therapy can occur especially when the drugs are stopped one at a time, are not taken correctly or omitted. It has been suggested that a 20% reduction in adherence to treatment may result in an 80% reduction in efficacy (Anderson *et al.,* 1998).

The high rate of replication that is found throughout the course of HIV infection and the variability of HIV, coupled with the relative inaccuracy of the enzyme HIV reverse transcriptase, are the main reasons for the frequent occurrence of copying errors in the transcription of viral genetic information. HIV replicates at the rate of around 10^8 to 10^{10} virus particles per day – depending on the viral load – probably giving rise daily to about $3x10^{-3}$ spontaneous changes (mutations) in its genetic sequence. The ultimate size of a viral population containing a mutation is probably determined by three concurrent factors: the forward mutation frequency, the replicative capability of the mutated virus and the "age" of the viral population containing the mutation, i.e. how long ago this population was generated. With the ongoing production of genetic variants of HIV there is then a continuous selection of the "fittest" virus population (WHO, 2002).

Sub-optimal ART regimens that allow replication of HIV to continue in the presence of antiretroviral drugs encourage the growth of viral populations that are carrying a genetic mutation which protects against these drugs. It is likely that many of these drug resistance mutations already exist before any antiretroviral drug is introduced and are further encouraged to proliferate under the selective pressure exerted by drug treatment.

Antiretroviral therapy can minimize the emergence of drug resistance in two ways:

- ⇨ By maximizing and sustaining the suppression of viral replication.
- ⇨ By using drugs where multiple mutations are required before resistance can occur (WHO, 2002).

2.4. Importance of Echocardiography to this Study

Cardiac complications in HIV infection were initially unrecognized or recognized late by clinicians because of a lack of awareness of cardiac disease in patients with AIDS and the tendency of cardiac disease to mimic clinically the far more common respiratory complications (Millei *et al.*, 1998). A review by Millei *et al.* (1998) deals with all the cardiac manifestations of AIDS and serves to highlight two problems and one indication. First of all, there are very few clinical studies. Current knowledge is based almost exclusively on echocardiography and autopsy studies. Observational or clinical trials based on syndromes of heart failure, tamponade and so forth would be useful. Secondly, there is very poor information on the impact of treatment. Clinicians have assumed that conventional treatment is appropriate for patients with heart failure and AIDS; however, there is some anecdotal evidence, based upon a low peripheral vascular resistance due to sepsis, that these patients tolerate angiotensin-converting enzyme inhibitors poorly. Finally, because cardiac complications are often clinically inapparent or subtle in the initial stages, periodic screening of HIV-positive patients by ECG and echocardiogram is probably indicated. The use of routine electrocardiography and echocardiography for asymptomatic patients with HIV infection is controversial (Millei *et al.*, 1998). Echocardiographic examination is more informative and sensitive than electrocardiographic monitoring.

Indeed, echocardiography appears to be the most appropriate way to detect heart involvement during HIV infection; it allows for early diagnosis and thus provides time to find the most suitable way of treating cardiac abnormalities, even in the early asymptomatic phase of the disease. Prompt recognition and treatment is important because palliative therapy with diuretics and vasodilators can be worthwhile: mild global left ventricular dysfunction appears to be reversible in many patients and a sub-group progresses to symptomatic heart failure without treatment. It appears prudent to perform a careful cardiac examination at the time of diagnosis of HIV to obtain a baseline functional assessment. An echocardiogram should be performed if there are signs of heart disease. One should note that the presence of left ventricular dysfunction on a single echocardiogram does not necessarily imply a poor prognosis. Ideally, viral load assays and CD4 cell counts should be used together with a careful clinical examination (Millei *et al.*, 1998; Warkentin, 1998). Millei *et al.* (1998) advised that an echocardiogram should be done every 6 months. Tavazzi (2002) agrees that the best diagnostic investigation par excellence (also in asymptomatic patients) is the echocardiogram. Lipshultz (1998) also recognizes the fact that cardiovascular abnormalities are common in HIV-infected patients, but are often difficult to diagnose clinically and are frequently attributed incorrectly to dysfunction in other organ systems. If these abnormalities are diagnosed early through screening, preventive and

therapeutic strategies for progressive left ventricular dysfunction can be applied (Lipshultz, 1998). HIV/AIDS related cardiomyopathy is a diagnostic challenge to which physicians should remain alert (Yunis *et al,* 1998).

Out of all the different studies and conlusions of the different authors, two strong agreements are clear. Firstly, that echocardiography is the best test to evaluate the HIV/AIDS patient's cardiac status, and secondly, that it is important to do screening echocardiographic tests from time to time to evaluate the cardiac status. Here some authors felt it is important to do a screening echocardiogram every six months, while others felt that it is more important to do the echocardiogram screening tests in the advanced stages of AIDS.

Chapter 3 Purpose and Objectives of Study

3.1. Purpose of the Study

Acquired immunodeficiency syndrome (AIDS) has become an increasingly important health problem worldwide (Acierno, 1989). The purpose of this research project is to focus on the influence of HIV/AIDS on the LVEF.

The stage of HIV disease as monitored by the CD4 cell count and the viral load is compared with the LVEF. The beneficial effect of ART on CD4 cell count and viral load is recognized and accepted. In this study the impact of ART on LVEF was evaluated, and the effect of the CD4 cell count and/or the viral load on the LVEF assessed. This was done by comparing the initial consultation together with its blood results and LVEF, to the different follow-ups.

The investigator compiled a pilot study in 2002 on 302 adult HIV/AIDS patients (after inclusion criteria but before exclusion criteria had been applied). These patients were also included (against the inclusion and exclusion criteria) in this research project. The pilot study showed a direct proportional relationship between the CD4 cell count and LVEF and an indirect proportional relationship between the viral load and the LVEF. It also showed that the prevalence of an LVEF which is below normal (<52%), occurred more often when the

CD4 cell counts were below 300 cells/ml and/or the viral loads were above 100 000 RNA copies/ml.

If this study gives approximately the same results as the pilot study it can bring medical practitioners, healthcare workers and medical aid schemes to new insights that can lead – for example – to:

Medical practitioners carefully considering an echocardiogram from time to time to evaluate the LVEF, especially when the viral load is above a certain value (according to the pilot study, a value of greater than 100 000cRNA copies/ml) and/or the CD4 cell count is below a certain value (according to the pilot study, a value of less than 300 cells/ml). This would highlight the importance of cardiovascular evaluation in patients with advanced HIV infection. It would have the benefit to patients of early detection of cardiac dysfunction and the institution of appropriate treatment.

The impact of antiretroviral therapy is positive in prolonging the lives of patients and improving the quality of their lives. By achieving full viral suppression, pathological processes that arise because of viraemia or immunological deficiency are minimized or eliminated. Since cardiac dysfunction is one of these processes, we should see a reduction of HIV associated cardiac disease when more and more people have access to antiretroviral therapy.

3.2. Study Objectives

The first part of the primary objective of this study was to show that there is a direct proportional relationship between the CD4 cell count and the LVEF and an indirect proportional relationship between the viral load and the LVEF. The second part of the primary objective of this study was to find a category for the CD4 cell count and viral load in which the prevalence of a lower than normal LVEF (≤52%), was higher.

The first part of the secondary objective was to see what the influence of antiretroviral therapy was on the CD4 cell count, viral load and the LVEF. The second part of the secondary objective was to see what the prevalence was of patients on antiretroviral therapy when the LVEF was below normal (≤52%) and CD4 cell count and viral load were within certain ranges

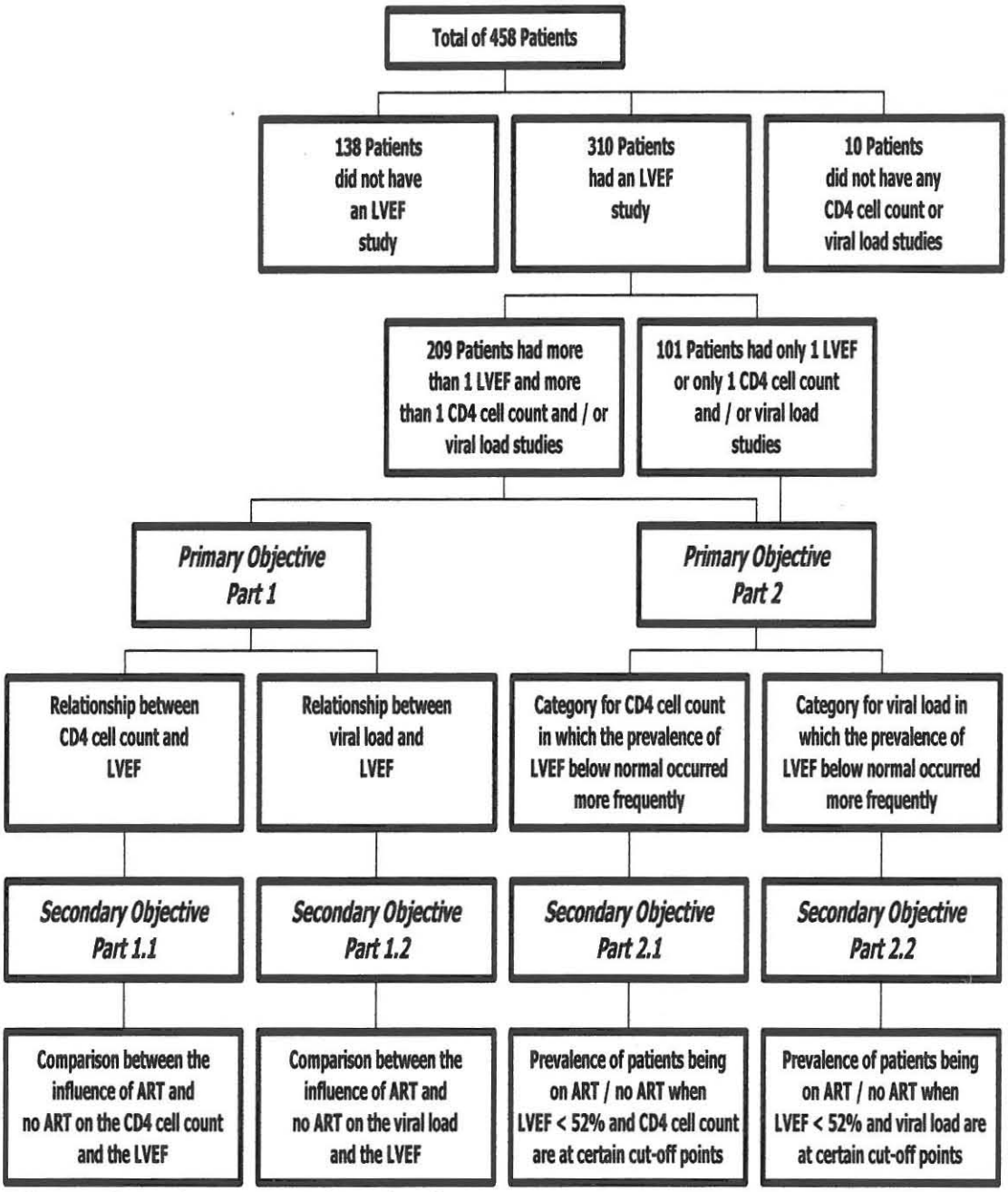

***Figure 3.1**: Summary of study objectives*

3.3. Hypothesis

There is a relationship between the viral load, CD4 cell count, and the LVEF of HIV/AIDS patients, such that when the viral load increases, the CD4 count declines and the LVEF decreases, sometimes below the normal value.

Effective antiretroviral therapy in HIV/AIDS patients will lead to a decrease in the viral load and an increase in the CD4 cell count. This will indirectly lead to an increase in the LVEF.

Chapter 4 Material and Methods

4.1. Study Design

This is an analytical design that compares the influence of the increase or decrease of the CD4 cell count and viral load on the LVEF of a patient, and the influence of ART on these variables. The initial consultation with its blood results and LVEF were compared to the follow-ups.

4.2. Study Population

4.2.1. Number of Subjects

A total of 458 patients were included after the inclusion criteria had been applied and the exclusion of other cardiac diseases and those who did not give consent were applied.

4.2.2. Infrastructure

Dr. M. Makotoko's private practice is very busy. She is a well-known specialist physician and cardiologist with a special interest, and wonderful success rate, in the treatment of HIV/AIDS patients. Many general practitioners, other physicians and patients refer HIV/AIDS patients to dr. Makotoko for treatment and advice. This results in the practice receiving patients from throughout South Africa and Lesotho.

4.2.3. Inclusion Criteria

- ⇨ HIV positive patients.
- ⇨ Of South African or Lesotho origin.
- ⇨ Any population group.
- ⇨ Between the ages of 18 – 85 years.
- ⇨ Male and Female.
- ⇨ Female patients: Both pregnant and non-pregnant women.
- ⇨ Patients who came for consultation to Dr Makotoko's surgery between March 1997 and 30 April 2003.
- ⇨ Patients who gave their consent.

4.2.4. Exclusion Criteria

- ⇨ HIV/AIDS patients who did not have an echocardiogram to evaluate the LVEF.
- ⇨ Those who did not give their consent.
- ⇨ Patients with other cardiac diseases that could influence the LVEF.
- ⇨ The variables of patients' data, which were compared to each other, and were not done within one month of each other, were excluded.

4.2.5. Justification for In- and Exclusion Criteria

For ethical reasons patients had to give informed consent before they could be included in the study.

The ethics committee of the University of the Free State reviewed the project proposal and gave authorisation.

All the population groups within South Africa and Lesotho were included in order to minimise possible bias on the basis of culture and demography. It is also important to include both South African and Lesotho citizens, because it is often difficult to distinguish between the two countries. A person often has Lesotho citizenship, but works and lives in South Africa and vice versa.

Patients with primary cardiac diseases were excluded as their diseases could influence the LVEF.

Patients who were included had to have their CD4 cell count, viral load and LVEF measurement done within a month of each other for accuracy of comparison because all the variables do fluctuate.

4.2.6. Subject Identification

The patients' file numbers – that the practice uses for account purposes – were used for identification within the study. These numbers were compiled using the first three letters of the surname of the person responsible for the account, followed by three numbers allocated by the computer account program (MASS®), followed by a slash (/), followed by a number indicating which dependent it was. For the purpose of publication, this number was linked to a numeric value. The numeric value was the only one used during this study. This was done for the confidentiality of the patient. Example: STE001/1 = nr. 100, the number 100 is the only one used during this study.

4.2.7. Withdrawal Criteria

Any patient could have withdrawn from this project at any stage. The patient's withdrawal would not have been held against him/her and his/her consultations and treatment with dr Makotoko would have continued as if he/she had never enrolled for the project.

During the course of this study no drop-outs occurred.

4.2.8. Pre-study Clinical Evaluation (Screening)

- ⇨ Patients received pre-test counselling from the cardiologist before an HIV test was performed.
- ⇨ Upon receipt of the results, patients received post test counselling.
- ⇨ Viral load and CD4 cell count tests were done.
- ⇨ The nature of the research project was discussed with the patient.
- ⇨ If the patient gave his/her consent, then an echocardiogram, for the evaluation of the LVEF, was done.
- ⇨ The patient and his/her data were weighed against the inclusion and exclusion criteria.

4.2.9. Drop-outs

There were no drop-outs during the course of this research project.

4.4. Biometric Plan

A statistical analyst from the Department of Biostatistics, University of the Free State, was consulted for assistance with the processing of the data. Variables were mostly described by frequencies and percentages. The relationship between the CD4 cell count and the LVEF (see table 5.1) as well as the relationship between the viral load and the LVEF (see table 5.2), were assessed by two-by-two tables. CD4 cell count and viral load were categorized according to certain cut-off points and the percentage cases with LVEF ≤52% and LVEF >52% were calculated. The percentage cases with below normal LVEF for each category of CD4 cell count and viral load were calculated and the percentages in the categories were compared with the 95% Wilson confidence intervals for the percentage difference (Altman *et al.*, 2001) (see Appendix A, Table A.1 and A.2). Cases were categorized as receiving no ART or any combination of ART and the percentages of cases with CD4 cell count and viral load below and above certain cut-off points were calculated. The percentages of cases with CD4 cell count and viral load below the cut-off point for each ART group were calculated and the percentages in the categories were compared with 95% Wilson confidence intervals for the percentage difference (Altman *et al.*, 2001) (see Appendix A, Table A.3 and A.4).

4.5. Practice Registration Form

All patients attending the practice for the first time completed the practice registration form. An example can be seen in Appendix B.2.

4.6. Subject Information and Informed Consent

Following pre- and post-test counseling, all HIV positive patients would be asked privately if they would be willing to be enrolled in the study. The purpose and format of the study as well as the financial implications, the consequences, the adverse effects and their right to withdraw without any negative effects on them or their doctor-patient relationship was explained thoroughly. Those that gave consent would then be requested to complete and sign the consent form. The patients' doctor, Dr. M. Makotoko, did all of this, before any study-related activities occurred. The Ethical Committee also approved the consent form (ETOVS 33/03). Both the patient information sheet and the consent form were available in English, Afrikaans and South Sotho (see Appendix B.1). The patients therefore had the choice of signing the consent form in any one of these languages.

4.7. Safety Variables

The research project was very safe. There were no adverse effects from the blood tests that were performed. Since the blood tests were not done for the sole purpose of the research project, the study itself had no adverse effects on the patient.

The echocardiogram, for the LVEF estimation, is totally harmless and no adverse effects were recorded during the course of this research project.

4.8. Premature Discontinuation of the Study

It did not become necessary to discontinue this research study prematurely. At no stage during the course of the research did the researcher or the study leaders feel that any patient's confidentiality was compromised or that any unethical procedures had occurred.

4.9. Accuracy of Data and Data Analysis

The researcher herself compiled the data from the patients' files. She personally double-checked all the data that were written down. This could not have been double-checked by someone else, since the patients' identities were still known at this stage.

All data entered into the computer and all statistical data that were counted manually were done and checked by the researcher herself. The processed data was also inspected for typing and/or counting errors by both Mr. DF Steenkamp (a registered Clinical Technologist in Cardiology) and Mrs. EH Thiele (a registered pharmacist).

The complete research project was read and approved by both the study leaders.

4.10. Good Clinical Practice (GCP) / Quality Assurance

All clinical work conducted under this research project was subjected to the GCP guidelines (Principles of ICH GCP).

The declaration of Helsinki's basic principle number 3 states that research should be conducted only by scientifically qualified persons and under the supervision of adequately qualified persons (World Medical Association Declaration of Helsinki, 2002). Therefore, the whole research project was compiled by a registered Clinical Technologist (Registered with the Health Professional Council of South Africa, number KT 0006165) under the supervision of two study leaders.

4.11. Confidentiality

The confidentiality of this study was of utmost importance. At no time during the research could the patient's identity be made known to any persons other than those to whom the patient had given his/her consent.

4.12. Ethics Committee

The study protocol and the informed consent form that were used in this study were submitted to the Ethics Committee of the University of the Free State and its approval was obtained. The Ethics Committee approved the study and supplied an ETOVS number, 33/03, to the researcher.

4.13. Apparatus

The echocardiographic machine that was used for the determination of the LVEF was the Toshiba CoreVision® using a 2.5MHz transducer.

Voigt and Partners – a pathology laboratory situated in Bloemfontein – did the CD4 cell count and viral load tests.

4.14. Measurement Techniques

4.14.1. Viral load test

Performed by: Voigt and Partners – a pathology laboratory in Bloemfontein, South Africa.

Purpose: Quantification of HIV. This is the best test for staging HIV disease and for monitoring the efficacy of treatment.

Tests: PCR (Polymerase Chain Reaction) - Roche Amplicor Technique

Sensitivity: Current generation tests measure from 20 - 50 RNA copies/ml and upwards.

Positive results: Patients should be serially monitored using the same laboratory technique. Results with PCR are about 1,8 times higher than results on the same sample using bDNA ISH [branched DNA (bDNA) in situ hybridization (ISH) as a method for detection of DNA and mRNA in whole cells (Kenny *et al.*, 2002)]; NASBA [nucleic acid sequence based amplification (Smart, 1996)] yields results about 10% higher than those obtained by PCR.

Precautions: Unexpectedly out-of-range results should be confirmed by repeat testing before clinical action is taken.

Appropriate sample: Blood in EDTA (purple-topped) tube (Miller, 2000a).

4.14.2. CD4 cell count

Performed by: Voigt and Partners – a pathology laboratory in Bloemfontein, South Africa.

Purpose: To determine the immunological status of the patient. It is an essential measure of the risk of contracting opportunistic infections and is thus used as an indicator for instituting disease prophylaxis.

Tests: Two techniques are used:

(a) Flow cytometry and

(b) Microcapillary fluorescence. Both yield comparable results.

Sensitivity: CD4 cells are measured relatively accurately when their number exceeds 50 cells/ml.

Precautions: Since CD4 cell counts can fluctuate due to extraneous factors, they should always be correlated with the CD4 cell % measurement.

True increases/decreases in cell % will be accompanied by corresponding increases/decreases in cell %. Single out-of-range results should be confirmed by re-testing.

Appropriate sample: Blood in EDTA (purple-topped) tube (Miller, 2000a).

Normal value: Between 510 and 1310 cells/ml (University of the Free State, 1997/8).

4.14.3. Estimation of LVEF

Performed by: A registered clinical technologist.

Purpose: To evaluate the left ventricle status of the heart. Measurements of the size of the left ventricle and the calculation of the LVEF (in %) are part of standard echocardiographic procedures.

Method: The LVEF was measured via echocardiographic methods. M-mode and two-dimensional echocardiography with colour Doppler and continuous wave (CW) Doppler was performed on the individuals in a left lateral decubitus position, using a Toshiba CoreVision® echocardiography machine using a 2.5MHz transducer. Images were taken orienting images at the two-dimensional mode on the transverse plane of the left ventricle.

The most frequently used method is with M-mode recording of the left ventricle cavity and wall thickness at the level of the mitral chords. The Teicholtz formula was used to calculate the LVEF. The Teicholtz formula is:

LVEF = [(end-diastolic^3 – end-systolic^3)/end-diastolic^3] x 100(%) (Echo by Web, 2003).

Sensitivity: The Teicholtz method is very accurate if there is no regional wall motion abnormality.

Results: An immediate result will be calculated right after the echocardiogram. Normal values are between 52% and 75% (Echo by Web, 2003). If the LVEF is lower than 52%, the results indicate left ventricular dysfunction.

Precautions: More than one M-mode recording of the left ventricle must always be done. If the LVEF measurements differ unexpectedly, the recording must be repeated. Patients can have poor echocardiographic images. In these cases the LVEF must be recorded with great care and it must be noted that the echocardiogram was technically difficult.

Colour Doppler and CW Doppler must be done to exclude any possible cardiac pathology that could influence the LVEF.

Time: A thorough echocardiogram will take approximately 15 - 20 minutes.

4.15. Method of Data Collection and Data Analysis

The researcher herself did all manual procedures.

- ⇨ Data of all the HIV/AIDS patients who visited the surgery from March 1997 up to 30 April 2003 were included.
- ⇨ As far as possible an echocardiogram, to determine the LVEF, was done on most of the patients who gave their consent.
- ⇨ The LVEFs were done according to the procedure explained under 4.14.3.
- ⇨ The CD4 cell count and the viral load tests were done at the pathology laboratory according to the procedures explained under 4.14.1. and 4.14.2.
- ⇨ After 30 April 2003, all the patients' files that contain all the clinical data, were drawn. A complete, detailed list of all the LVEF, viral load, CD4 cell count tests, type of ART and applicable dates were made. The whole echocardiogram report was inspected to see if the patient had any other

cardiac diseases that could influence the LVEF. This was also noted. All of these procedures were done manually.

- ⇨ All the data were counterbalanced against the exclusion and inclusion criteria. This was done manually. All the data that met the exclusion criteria were deleted.
- ⇨ All the data were printed out for manual analysis.
- ⇨ The researcher manually counted the total amounts of each relationship between the variables.

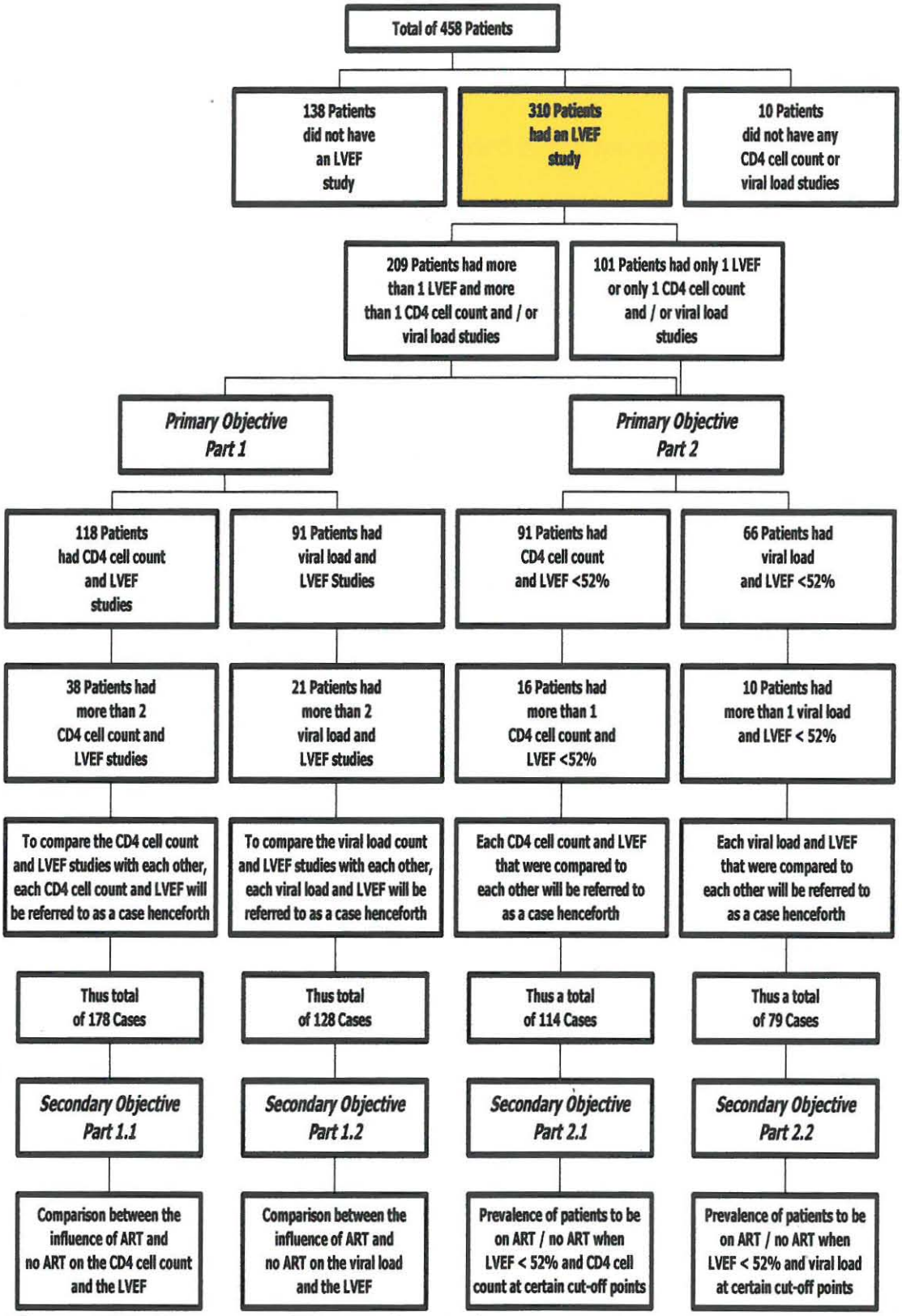

Figure 5.1: *Summary of the study population.*

5.1. General

A total of 458 patients were included in the research project after the exclusion and inclusion criteria had been met. 138 patients did not have an LVEF study for reasons discussed under 6.1. They were excluded. Ten patients did not have any CD4 cell count or viral load tests done, usually due to financial reasons, or because patients passed away before these tests could be done, or because they did not return after their initial visit for follow-up (see figure 5.1). There were 310 patients left. In this group, 165 patients were female and 145 patients were male, with ages ranging between 20 – 70 years (average age was 45 years).

With reference to figure 5.1, 209 patients had more than one LVEF and CD4 cell count that could be compared to each other. To accommodate this situation, we referred to each follow-up as a case. Therefore, one patient could have multiple cases that were compared. 101 patients had only one LVEF or only one CD4 cell count or viral load study.

5.2. Comparison between the CD4 cell count and the LVEF.

Out of the 118 patients who had CD4 cell counts and LVEF studies to compare to each other, 38 patients had two or more CD4 cell counts and LVEF studies. This resulted in a total of 178 cases of LVEF studies and CD4 cell counts to be compared (see figure 5.1).

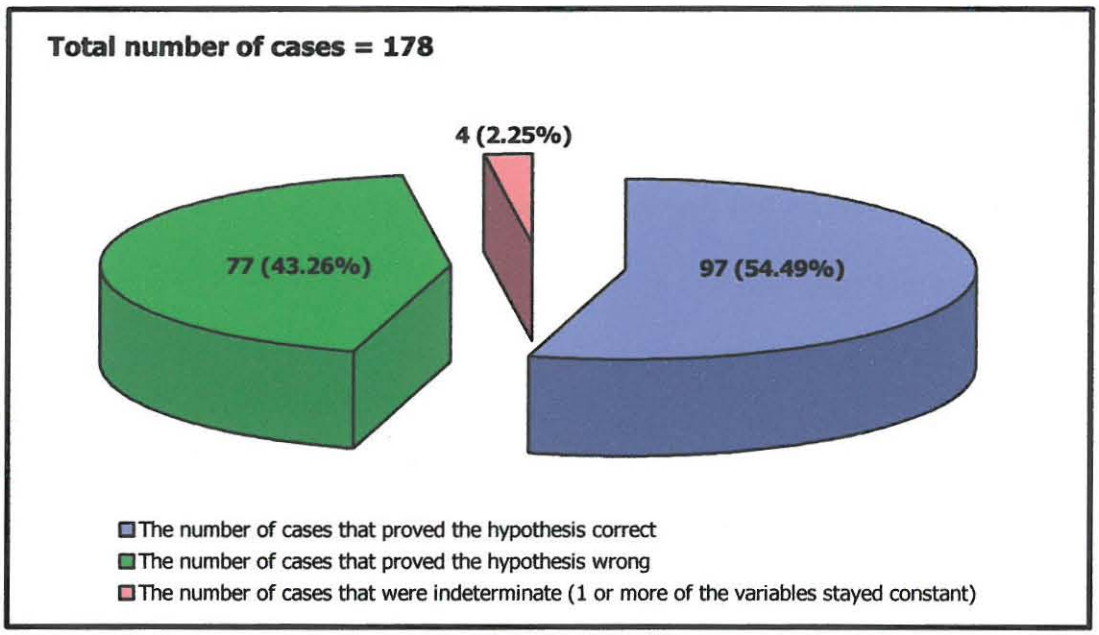

Figure 5.2: *Comparison between the CD4 cell count and the LVEF of consecutive follow-ups.*

LVEF: Left ventricle ejection fraction (Normal value:>52% - 75%)

Table 5.1: *Comparison between the CD4 cell count and the LVEF of consecutive follow-ups.*

CD 4 cell count	LVEF ↓	LVEF ↑	LVEF Constant	LVEF *Total*
↓	29	30	3	62
↑	47	68	0	115
Constant	0	1	0	1
Total	76	99	3	178

↑Indicates an increase; ↓Indicates a decrease.
LVEF: Left ventricle ejection fraction (Normal value:>52% - 75%)
Calculations:

- *Direct proportional relationship between the CD4 cell count and LVEF:*
  29 + 68 = 97 ÷ 178 x 100 = 54.49%
- *Indirect proportional relationship between the CD4 cell count and LVEF:*
  47 + 30 = 77 ÷ 178 x 100 = 43.26%
- *Indeterminate relationship between the CD4 cell count and LVEF (one or more of the variables stayed constant):*
  0 + 1 + 3 + 0 + 0 = 4 ÷ 178 x 100 = 2.25%

This part of the primary objective focused on the relationship between the CD4 cell count and the LVEF. Figure 5.1 shows that out of the 118 patients who had CD4 cell counts and LVEF studies that could be compared, 38 patients had more than two CD4 cell counts and LVEF studies. To compare the CD4 cell counts and LVEF studies, each CD4 cell count and LVEF study that could be compared to each other was referred to as a case. There were 178 cases in which the CD4 cell counts and LVEF studies could be compared. Figure 5.2 and table 5.1 show that out of the 178 cases, 97 cases (54.49%) showed a direct proportional relationship between the CD4 cell count and the LVEF. 77 cases (43.26%) showed an indirect proportional relationship between the CD4 cell count and the LVEF. Four cases (2.25%) showed an indeterminate relationship between the CD4 cell count and the LVEF, due to one or more of the variables that stayed constant and there was no definite

relationship between the variables that stayed constant. In one case the CD4 cell count stayed constant at 199 cells/ml, in the other three cases the LVEF stayed constant at 58%, 27% and 64% respectively.

5.3. Comparison between the viral load and the LVEF

Out of the 91 patients who had viral load studies and LVEF studies to compare to each other, 21 patients had two or more viral load studies and LVEF studies. This resulted in a total of 128 cases of LVEF studies and viral load studies to be compared (see figure 5.1).

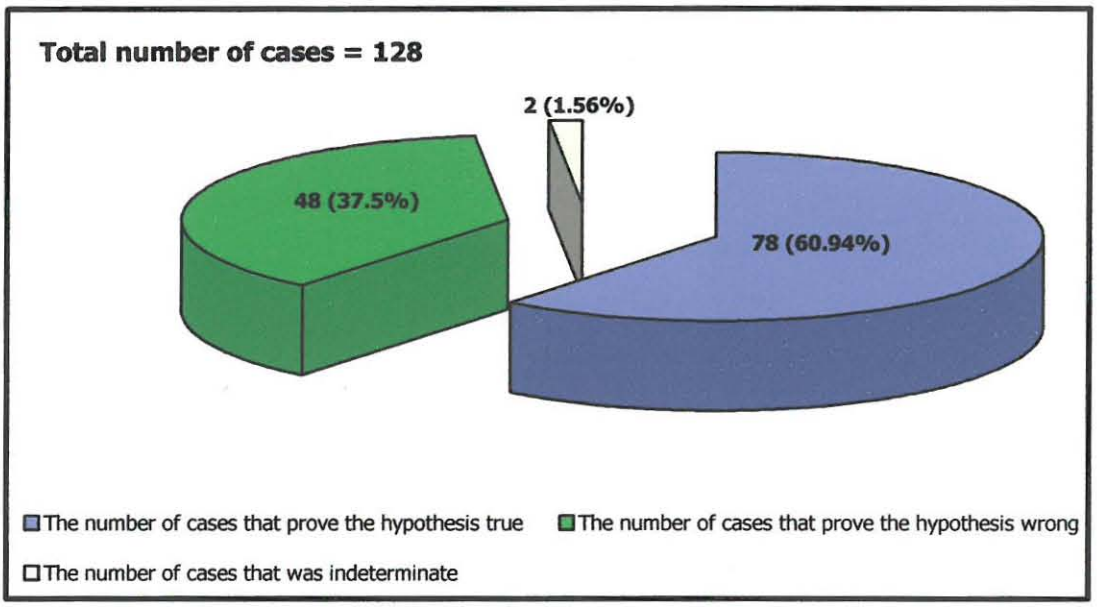

Figure 5.3: *Comparison between the viral load and the LVEF of consecutive follow-ups.*

LVEF: Left Ventricle ejection fraction (Normal value: >52% - 75%)

Table 5.2: *Comparison between the viral load and the LVEF of consecutive follow-ups.*

Viral Load (RNA copies/ml)		LVEF (%) ↓	LVEF (%) ↑	LVEF (%) Constant	*Total*
↓		20	42	0	62
↑		19	15	2	36
Constant	≥750 000	3	3	0	30
Constant	≤400	10	14	0	
Total		52	74	2	128

↑Indicates an increase; ↓Indicates a decrease.
LVEF: Left Ventricle ejection fraction (Normal value: >52% - 75%)
Calculations:

- ⇨ *Direct proportional relationship between the viral load and LVEF:*
  42 + 19 = 61 ÷ 128 x 100 = 47.66%
- ⇨ *Indirect proportional relationship between the viral load and LVEF:*
  20 + 15 = 35 ÷ 128 x 100 = 27.34%
- ⇨ *Relationship where one of the variables stayed constant:*
  a) *Relationships that agree with hypothesis:*
  3 + 14 = 17 ÷ 128 x 100 = 13.28%
  b) *Relationships that disagree with hypothesis:*
  10 + 3 = 13 ÷ 128 x 100 = 10.16%
  c) *Relationships that neither agree nor disagree with hypothesis, i.e. indeterminate relationships:*
  2 ÷ 128 x 100 = 1.56%

This part of the primary objective focused on the relationship between the viral load and the LVEF. Figure 5.1 shows that out of the 91 patients who had viral load and LVEF studies that could be compared, 21 patients had more than two viral load studies and LVEF studies. To compare the viral load studies and LVEF studies, each viral load and LVEF study that could be compared to each other was referred to as a case. There were 128 cases in which the viral load and LVEF studies could be compared. Table 5.2 show that out of the 128 cases, 61 cases (47.66%) had an indirect proportional relationship between the viral load and the LVEF. 35

cases (27.34%) showed a direct proportional relationship between the viral load and the LVEF. There were 32 cases where one of the variables stayed constant. Out of these 32 cases there where 14 cases (10.94%) where the viral load stayed constant below 400 RNA copies/ml and the LVEF increased. This also shows a positive correlation with the hypothesis, since the goal is to keep an HIV/AIDS patient's viral load below 400 RNA copies/ml and the hypothesis implied that when the viral load is improving (i.e. decreasing), the LVEF will also increase. There were three cases (2.34%) that also agreed with the hypothesis, this was when the viral load stayed constant above 750 000 RNA copies/ml and the LVEF decreased. Out of the total of 32 cases where one of the variables stayed constant, there were two relationships that disagreed with the hypothesis. One relationship was where the viral load of 10 cases (7.81%) stayed constant below 400 RNA copies/ml, but despite the ideal viral load, the LVEF decreased. The other relationship was where the viral load of three cases (2.34%) stayed constant above 750 000 RNA copies/ml, but the LVEF increased. These two relationships do not agree with the hypothesis that stated that when the viral load is improving (i.e. decreasing, with the ideal value of <400 RNA copies/ml), the LVEF would also improve. The last part of the 32 cases where one of the variables stayed constant was where two cases (1.56%) had an LVEF that stayed constant, while the viral load increased. These two cases were indeterminate, because there was no definite relationship between the two cases (see figure 5.3).

To summarize the findings of figure 5.3 and table 5.2, there were 60.94% of cases that showed the hypothesis true, while 37.5% showed the hypothesis wrong and 1.56% of cases were indeterminate.

5.4. Percentage of cases with LVEF ≤52% in which the CD4 cell count was within a specific range.

Out of the 310 patients who had CD4 cell counts, irrespective of the number of cases per patient, 91 patients had a below normal LVEF. Sixteen of these patients had more than one case per patient. This resulted in 114 cases of patients with CD4 cell counts and LVEF studies where the LVEF study was below normal (≤52%), and 337 cases of patients with CD4 cell counts and LVEF studies where the LVEF study was within the normal range (>52% - 75%) (see figure 5.1). This results in a total of 451 cases (see Appendix A, Table A.1, Part 1).

The cut-off points for the CD4 cell counts were 50, 100, 150, 200, 250, 300, 350, >350 cells/ml. The >350 cells/ml cut-off point (i.e. 0 - >350) included all the cases defined above (see Appendix A, Table A.1, Part 1 – 8).

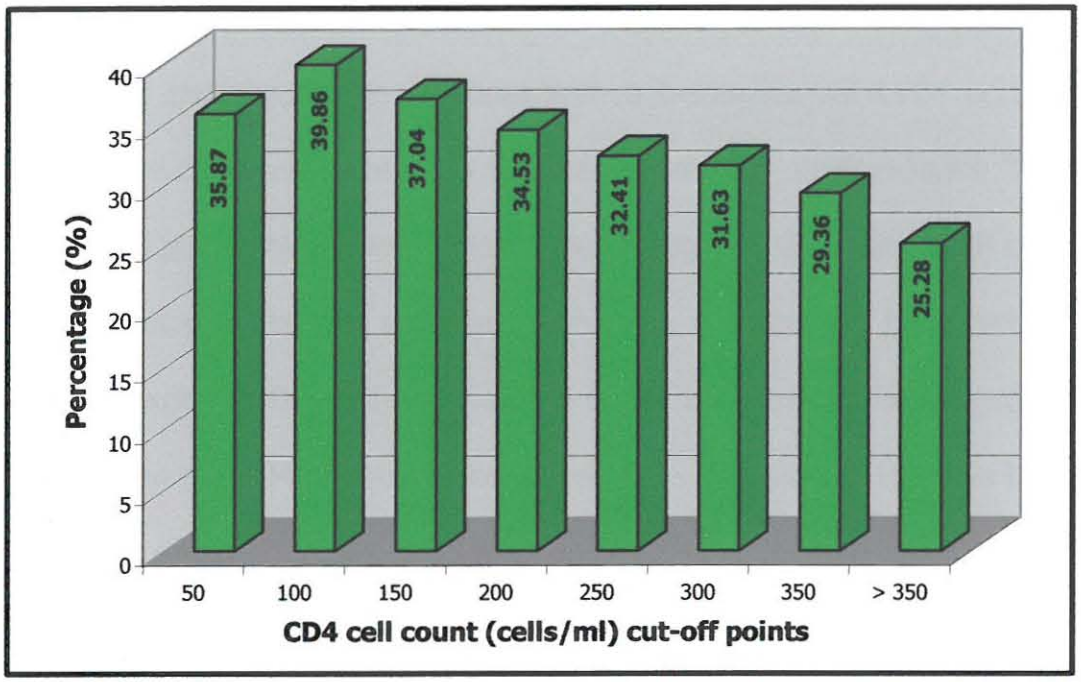

Figure 5.4: *Percentage of cases with LVEF below normal (≤52%), when the CD4 cell count was less or equal to specific cut-off points.*
LVEF: Left Ventricle ejection fraction (Normal value: >52% - 75%)

Here the focus was to see what the prevalence of a below normal LVEF (≤52%) was when the CD4 cell count was at certain cut-off points. Out of the 310 patients who had CD4 cell counts and LVEF studies, irrespective of the amount of cases per patient, 91 patients had an LVEF of below normal (≤52%). Sixteen of these patients had more than one case per patient. This resulted in 114 cases that could be investigated. There were 219 patients with normal LVEF (>52%) (see figure 5.1). These 219 patients made up a total of 337 cases (see Appendix A, Table A.1, Part 1). Therefore the total number of cases in which the CD4 cell count and LVEF (both normal and below normal values) could be compared was 451 cases.

From figure 5.4 (see Appendix A, Table A.1, Part 1 – 8) one can see that the percentage of cases with a below normal LVEF occurred more frequently when the CD4 cell count was ≤100 cells/ml. There was also a steady decline in the percentage of cases with LVEF below normal as the CD4 cell count increased. Of note was the small, but unexpected fall in the prevalence of the LVEF below normal (≤52%) when the CD4 cell count was between 0 – 50 cells/ml. Here we had a percentage of cases of 35.87% as opposed to 39.86% when the CD4 cell count was at the cut-off point of 100 cells/ml. The difference in the percentage of cases with below normal LVEF between cases with ≤100 cells/ml and ≤50 cells/ml was 3.99%.

Figure 5.5 shows that the 95% Wilson confidence interval for the percentage difference was of statistical importance at all the abovementioned cut-off points for the CD4 cell count when the LVEF was below normal, since the number zero (0) was not included in any of 95% confidence intervals at any cut-off points during this study. The most significant 95% confidence interval was found in the percentage of cases in which the CD4 cell count was ≤100 cells/ml.

(See Appendix A, Table A.1, Part 1 – 8)

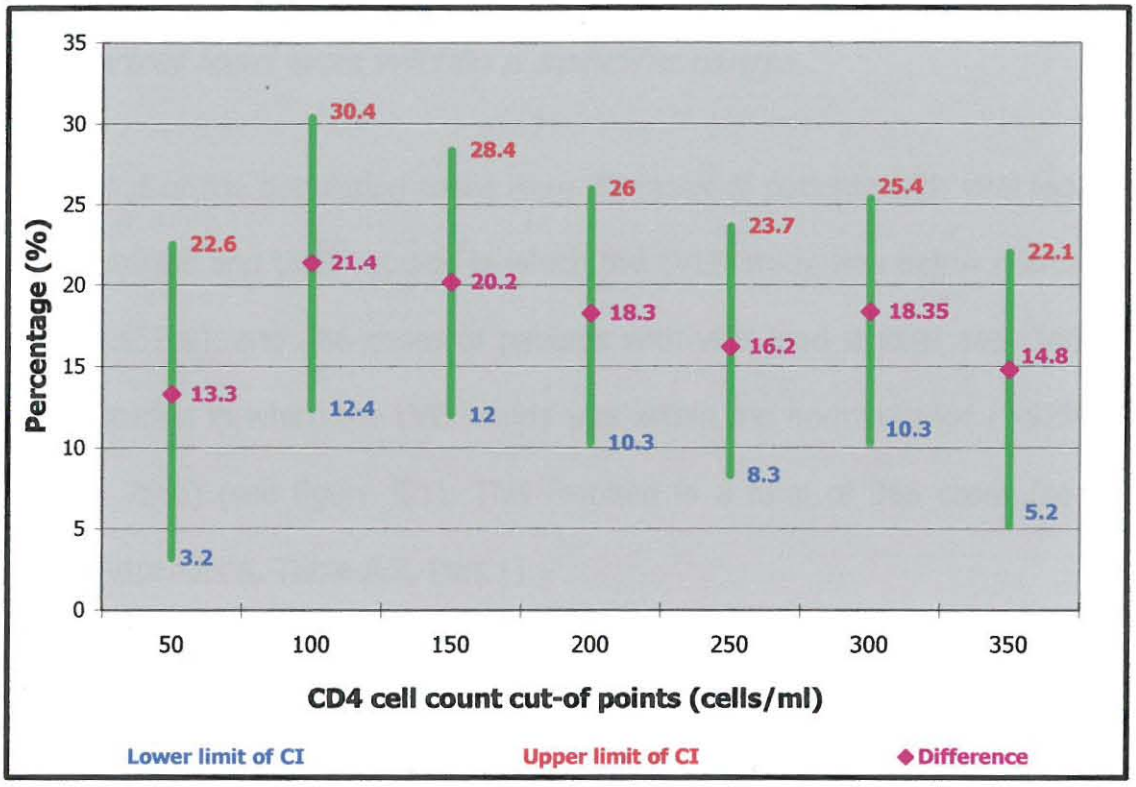

Figure 5.5: *Graphic presentation of the 95% Wilson confidence interval of the percentage difference at certain CD4 cell count cut-off points, when the LVEF was below normal (≤52%).*

LVEF: Left Ventricle ejection fraction (Normal value: >52% - 75%).
CI: 95% Wilson confidence interval of the percentage difference.
The difference in the percentages was calculated by the difference between the percentage of cases with below normal LVEF in cases with CD4 cell count less or equal to (≤) the cut-off point, and the CD4 cell count larger than the cut-off point.

5.5. Percentage of cases with LVEF ≤52% in which the viral load was within a specific range.

Out of the population there were 79 cases of patients with viral load studies and LVEF studies in which the LVEF study was below normal (≤52%), and 286 cases of patients with viral load studies and LVEF studies in which the LVEF study was within the normal range (>52% - 75%) (see figure 5.1). This resulted in a total of 365 cases (see Appendix A, Table A.2, Part 1).

The ranges in which the viral load (PCR-method) can be measured, are between <400 RNA copies/ml (in some cases when the doctor requested the ultra sensitive HIV PCR viral load test, then the lowest limit is <50 RNA copies/ml) up to >750 000 RNA copies/ml. The cut-off points in this study were ≤1 000 (i.e. $\leq 10^3$), 10 000 (i.e. 10^4), 100 000 (i.e. 10^5) and 1 000 000 (i.e. 10^6). Therefore the lowest value of viral load (<400 RNA copies/ml or <50 RNA copies/ml in some cases) was included in the ≤1 000 cut-off point, and the highest value of viral load (≥750 000 RNA copies/ml) was included in the cut-off point of <1 000 000. The cut-off point of <1 000 000 will therefore include all the above defined cases (i.e. 0 – 1 000 000) (see Appendix A, Table A.2, Part 1 – 4).

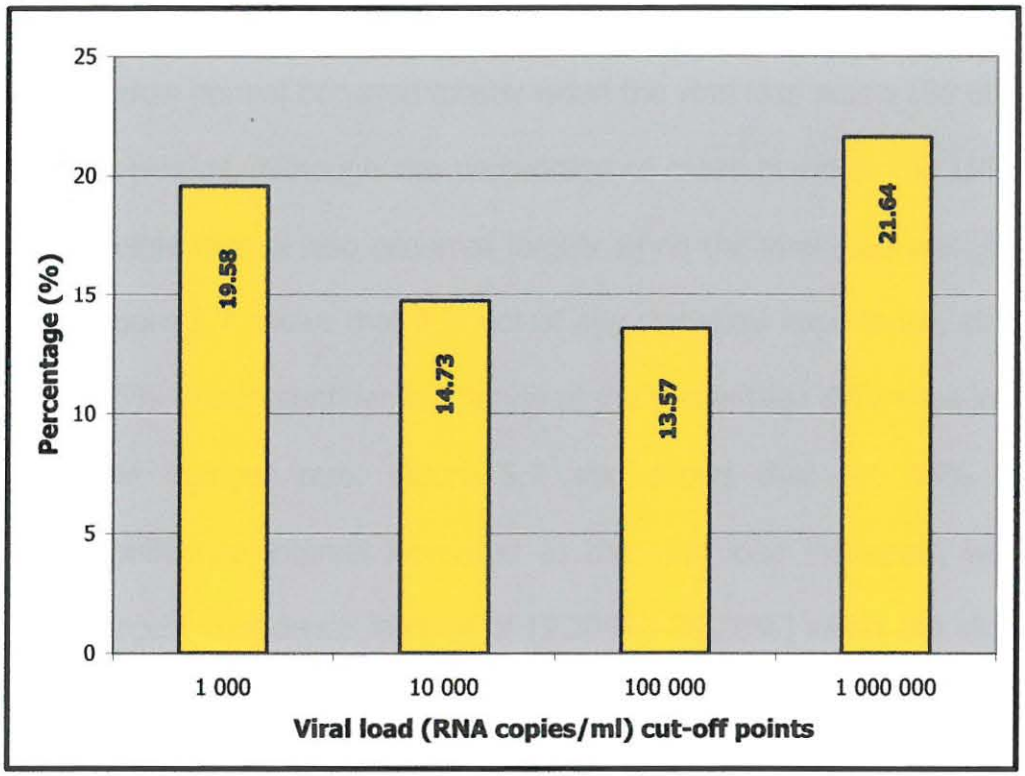

Figure 5.6: *Percentage of cases with below normal (≤52%) LVEF in cases with viral load below or equal to the cut-off points.*

LVEF: Left ventricle ejection fraction (normal value: >52% - 75%)

Here the focus was to see what the prevalence of a below normal LVEF (≤52%) was when the viral load was at certain cut-off points. Out of the 310 patients who had viral load and LVEF studies, irrespective of the number of cases per patient, 66 patients had an LVEF of below normal (≤52%). 10 of these patients had more than one case per patient. This resulted in 79 cases that could be investigated. There were 244 patients with normal LVEF (>52%). These 244 patients had a total of 286 cases. Therefore the total number of cases in which the viral load and the LVEF (both normal and below normal values) could be compared was 365 (see Appendix A, Table A.2, Part 1).

(See Appendix A, Table A.2, Part 1 – 4).

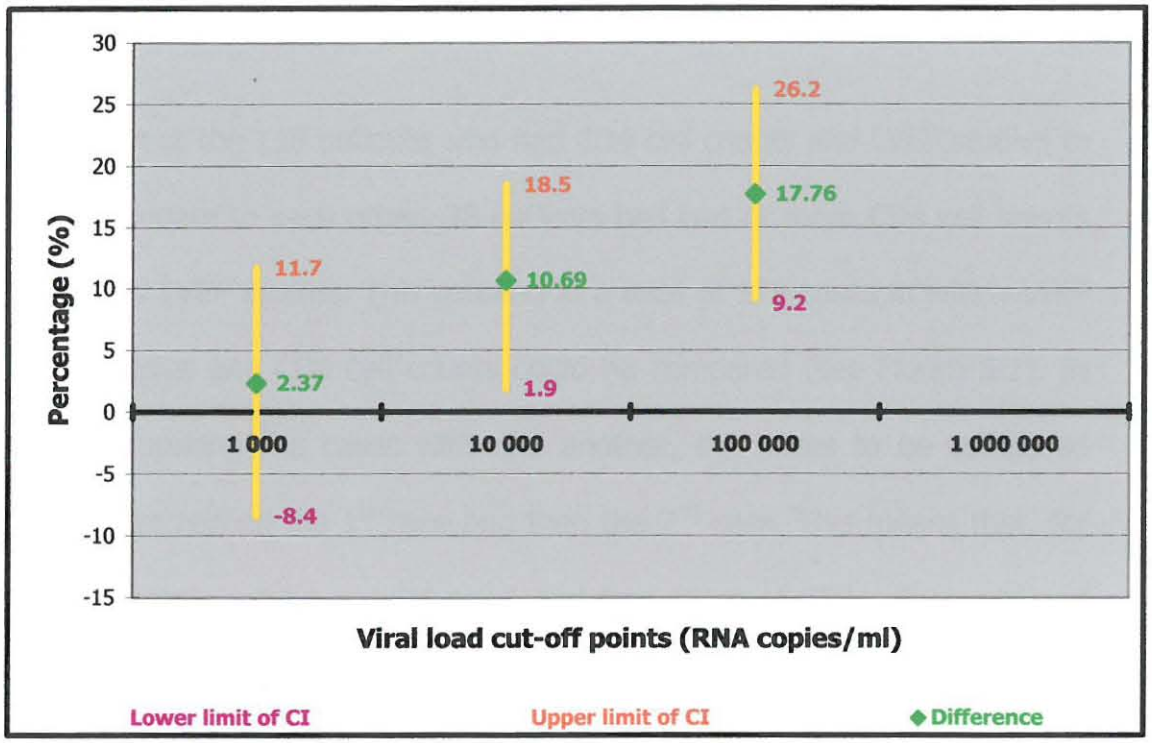

Figure 5.7: *Graphic presentation of the 95% Wilson confidence interval of the percentage difference for certain viral load cut-off points in which the LVEF was below normal (≤52%).*

The difference in the percentages of cases was calculated by the difference between the percentage of cases with below normal LVEF in cases with viral load larger than the cut-off point and the viral load less or equal to (≤) the cut-off point.
LVEF: Left ventricle ejection fraction (normal value: >52% - 75%)
CI: 95% Wilson confidence interval of the percentage difference.

5.6. Influence of ART on the CD4 cell count and the LVEF.

Out of the 118 patients who had CD4 cell counts and LVEF studies to compare to each other, 38 patients had two or more CD4 cell counts and LVEF studies. This resulted in a total of 178 cases in which LVEF studies and CD4 cell counts could be compared (see Figure 5.1). In comparing the cases with one another, the cases to be compared were named the 1st case and then the 2nd case. This means that, for example, where patient no. 1 had four cases of CD4 cell counts and LVEFs to compare, the comparison would be done like this:

30 January 2001	CD4 = 40,	LVEF =50%, No ART	*1st No ART, **2nd No ART
20 March 2001	CD4 = 100,	LVEF = 60%, No ART	*1st No ART, **2nd Any combination ART
30 July 2001	CD4 = 200,	LVEF = 65%, ART	*1st Any combination ART, **2nd Any combination ART
30 September 2001	CD4 = 350,	LVEF = 68%, ART	

** 1st means the patient was at the first set of variables that was compared to a 2nd set of variables on No ART/Any combination of ART.*
*** 2nd means the patient was at the second set of variables that was compared to the first set of variables on No ART/Any combination of ART.*

Table 5.3: *Summary of the influence of ART on the CD4 cell count and the LVEF.*

		Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	
		CD4↑ LVEF↑	CD4↑ LVEF↓	CD4↓ LVEF↓	CD4↓ LVEF↑	CD4(c) LVEF↑	CD4↓ LVEF(c)	*Total*
		Frequency Row % Column % %	Frequency Row % Column % %	Frequency Row % Column % %	Frequency Row % Column % %	Frequency Row % Column % %	Frequency Row % Column % %	
Row 1	*1st No ART **2nd No ART	13 27.08 19.11	7 14.58 14.89	12 25 41.38	16 33.33 53.33	0 0 0	0 0 0	48
Row 2	1st No ART 2nd Any combination of ART	26 54.17 38.24	13 27.08 27.66	4 8.33 13.79	5 10.42 16.67	0 0 0	0 0 0	48
Row 3	1st Any combination of ART 2nd No ART	0 0 0	0 0 0	2 100 6.9	0 0 0	0 0 0	0 0 0	2
Row 4	1st Any combination of ART 2nd Any combination of ART	29 36.25 42.65	27 33.75 57.45	11 13.75 37.93	9 11.25 30	1 1.25 100	3 3.75 100	80
	Total	68	47	29	30	1	3	178

↑ Indicates an increase; ↓ Indicates a decrease; (c) indicates a variable that stayed constant

LVEF: Left ventricle ejection fraction (Normal value:>52% - 75%)

ART: Antiretroviral therapy.

** 1st means the patient was at the first set of variables that was compared to a 2nd set of variables on No ART/Any combination of ART.*

*** 2nd means the patient was at the second set of variables that was compared to the first set of variables on No ART/Any combination of ART*

/ Ex: On 13 January 2003 the patient's CD4 count was 15 and the LVEF was 52% and at this stage the patient was on no ART; on 13 June 2003 the patient came for a follow-up. His CD4 cell count was 150 and the LVEF was 60% and the patient was at this stage on any combination of ART. Then the CD4 cell count increased, the LVEF increased, while the patient went from 1st No ART to 2nd any combination of ART.*

This first part of the secondary objective focused on the influence that ART (antiretroviral treatment) had on the CD4 cell count and the LVEF (both normal and below normal values). With reference to figure 5.1, 209 patients had more than one LVEF and CD4 cell count that could be compared to each other. To accommodate this situation in which one patient could have more than one follow-up visit to compare, we referred to each follow-up that was compared, as a case. Therefore, one patient could have multiple cases that were compared. 101 patients had only one LVEF or only one CD4 cell count or viral load study. Out of the 118 patients who had CD4 cell counts and LVEF studies that could be compared, 38 patients had more than two CD4 cell counts and LVEF studies. To compare the CD4 cell counts and LVEF studies, each CD4 cell count and LVEF study that could be compared to each other was referred to as a case. There were 178 cases in which the CD4 cell counts and LVEF studies could be compared.

During this discussion, when "1st and 2nd" are referred to, it means that one patient came for more than one follow-up. These follow-ups were compared to each other to see if the CD4 cell count, LVEF and viral load increased, decreased or stayed constant from one visit to another, as well as to see whether the patient was on any combination of ART or on no ART from one follow-up to another. Therefore, "1st" referred to the first set of variables that was

compared to the following set of variables (at the second follow-up), which was named "2nd".

This part of the results will be discussed as figures 5.8 and 5.9. In figure 5.8 the column percentages from table 5.3 were compared to one another. This means that the percentage of cases that were on any combination of ART or on no ART (rows in table 5.3) from one visit to another was compared to different relationships between the CD4 cell count and the LVEF (columns in table 5.3). This was done to see what the influence was of ART on the total number of cases in which there was a specific relationship between the CD4 cell count and the LVEF. On the other hand, figure 5.9 shows the horizontal row percentages from table 5.3 which were compared to each other. This means that the percentage of cases that were on any combination of ART or on no ART (rows in table 5.3) from one visit to another was compared to different relationships between the CD4 cell count and the LVEF (columns in table 5.3). This was done to see what the percentage of cases with certain relationships between the CD4 cell count and the LVEF was when the cases were on any combination of ART or on no ART from one visit to another.

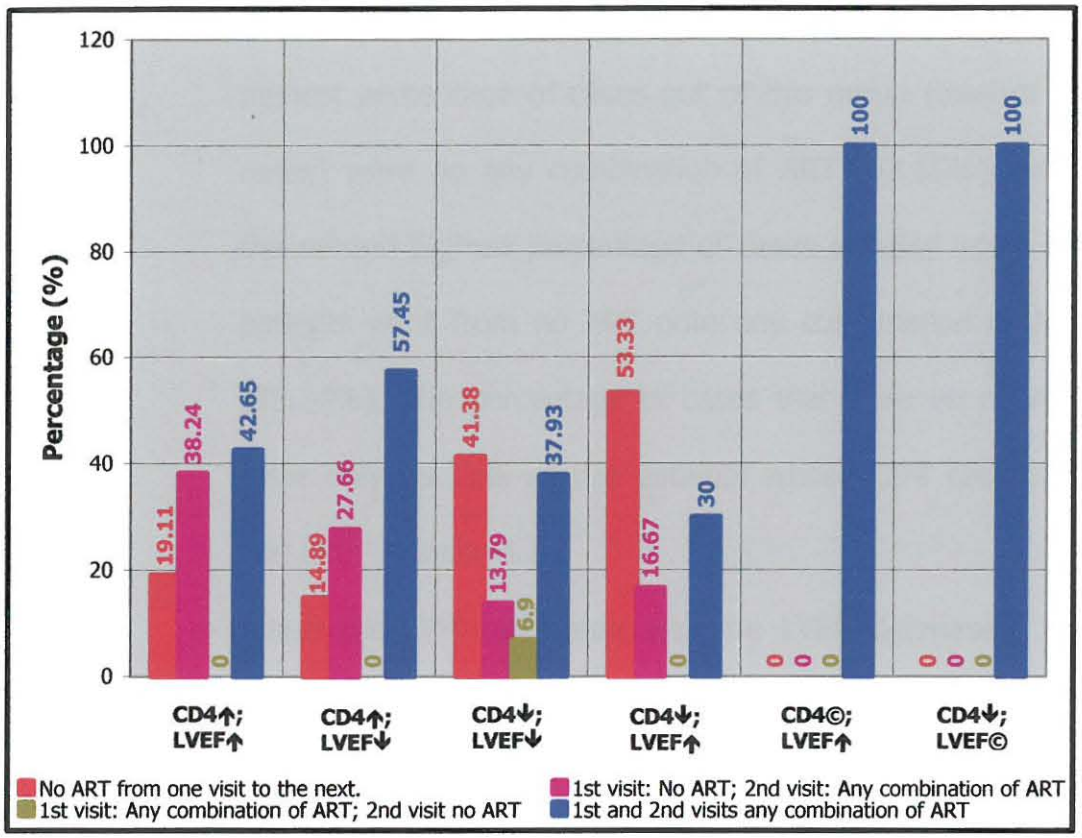

Figure 5.8: *Summary of the influence of ART on the CD4 cell count and LVEF from one visit to another (Column percentages – see table 5.3).*

© indicates a variable that stayed constant
↑Indicates an increase; ↓Indicates a decrease.
LVEF: Left ventricle ejection fraction (Normal value:>52% - 75%)
1st means the patient was at the first set of variables that was compared to a 2nd set of variables on No ART/Any combination of ART.
2nd means the patient was at the second set of variables that was compared to the first set of variables on No ART/Any combination of ART
Ex: On 13 January 2003 the patient's CD4 count was 15 and the LVEF was 52% and at this stage the patient was on no ART; on 13 June 2003 the patient came for a follow-up. His CD4 cell count was 150 and the LVEF was 60% and the patient was at this stage on any combination of ART. Then the CD4 cell count increased, the LVEF increased, while the patient went from 1st No ART to 2nd on any combination of ART.

In figure 5.8 the direct proportional relationship between the CD4 cell count and the LVEF was investigated. There were two different direct proportional relationships between the CD4 cell count and the LVEF, namely when the CD4 cell count increased and the LVEF increased, and secondly when the CD4 cell count decreased and the LVEF decreased. For these two relationships figure 5.8 shows the following:

- ⇨ When the CD4 cell count and the LVEF increased, the highest percentage of cases out of this group (total of 68 cases) were on any combination of ART (42.65%), while the second highest percentage of cases occured when the patients went from no ART onto any combination of ART (38.24%). The percentage of cases that were on no ART were only 19.11% of the patients whose CD4 cell count and LVEF increased.
- ⇨ When the CD4 cell count and the LVEF decreased, the highest percentage of cases out of this group (total of 29 cases) were on no ART (41.38%). The second highest point was for those patients who were on any combination of ART (37.93%). Those cases that were at first on no ART and then went onto ART, amounted to 13.79%, while those cases that were at first on some sort of combination of ART and then stopped (i.e. no ART) only came to 6.90%.

The indirect proportional relationship between the CD4 cell count and the LVEF in figure 5.8 shows two possible relationships, namely the CD4 cell count increased, while the LVEF decreased, and CD4 cell count decreased while the LVEF increased. The following could be seen from figure 5.8:

- ⇨ When the CD4 cell count increased and the LVEF decreased (total of 47 cases), the highest percentage of cases were

on any combination of ART (57.45%), while the second highest point was for those cases that were at first on no ART, but went onto ART before the second follow-up visit (27.66%). The two lowest points were for those cases that were on no ART (14.89%) and those who were at first on ART, but then stopped before the second follow-up visit (0%).

- ⇨ When the CD4 cell count decreased and the LVEF increased (total of 30 cases), the highest percentage of cases occurred when they were on no ART from one visit to another (53.33%), with those who were on any combination of ART on both visits making up 30%. The two lowest points were for those that were on no ART at first, but began ART before the next visit (16.67%), and the lowest point was those who were on ART at first, but stopped the ART. This was 0%.

There were cases which showed an indeterminate relationship between the CD4 cell count and the LVEF where one of the variables stayed constant. Figure 5.8 shows the following:

- ⇨ When the CD4 cell count stayed constant and the LVEF increased (total of one case), the percentage of cases that were on ART with both visits, was 100%. It is important to note that in this relationship between the CD4 cell count

and the LVEF, there was only one case. Therefore the very high peak of 100%.

⇨ When the CD4 cell count decreased, while the LVEF stayed constant (total of three cases), the percentage of cases that were on any combination of ART, was the highest with 100%. It is important to note that in this relationship between the CD4 cell count and LVEF, there were only three cases and all three cases were on ART. Therefore the very high peak of 100%.

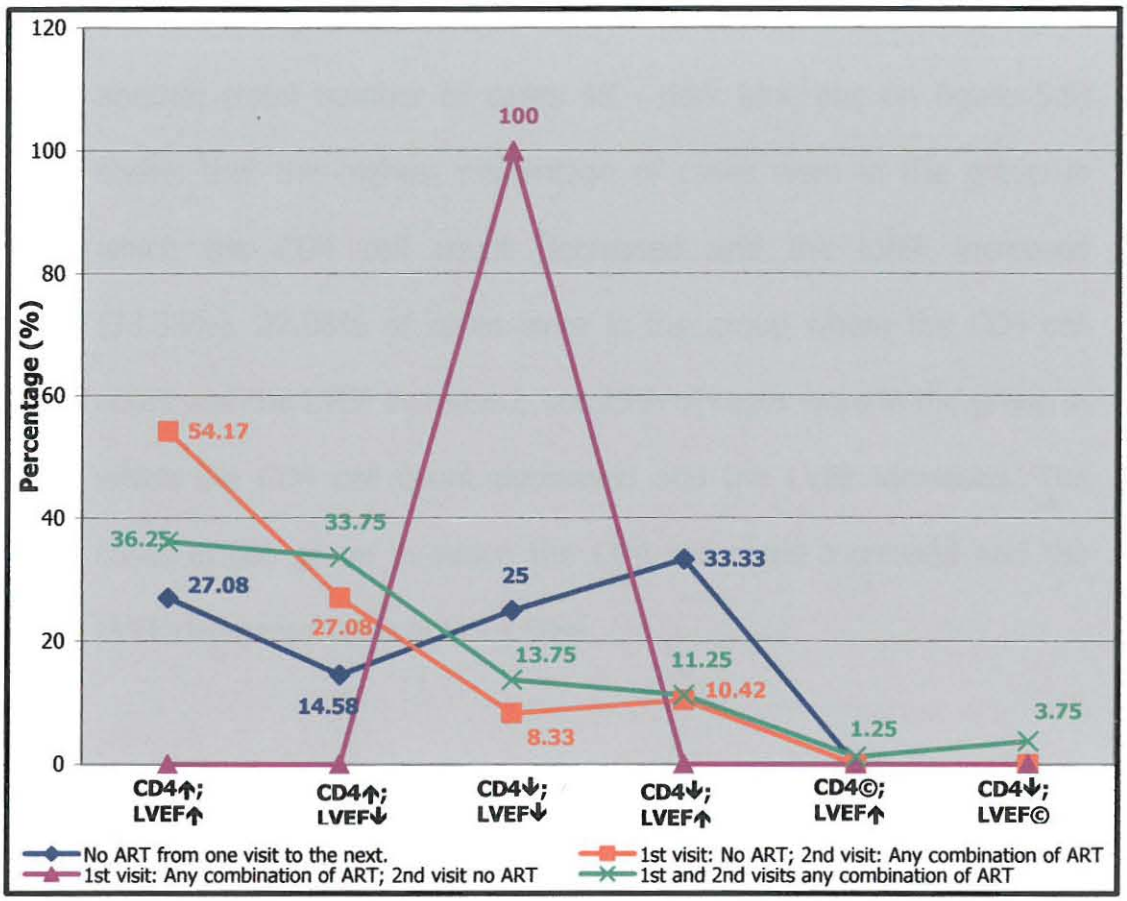

Figure 5.9: *Summary of the influence of ART on the CD4 cell count and LVEF from one visit to another (Row percentages – see Table 5.3).*

© Indicates a variable that stayed constant
↑Indicates an increase; ↓Indicates a decrease.
LVEF: Left ventricle ejection fraction (Normal value:>52% - 75%)
ART: Antiretroviral therapy
1st means the patient was at the first set of variables that was compared to a 2nd set of variables on No ART/Any combination of ART.
2nd means the patient was at the second set of variables that was compared to the first set of variables on No ART/Any combination of ART
Ex: On 13 January 2003 the patient's CD4 count was 15 and the LVEF was 52% and at this stage the patient was on no ART; on 13 June 2003 the patient came for a follow-up. His CD4 cell count was 150 and the LVEF was 60% and the patient was at this stage on any combination of ART. Then the CD4 cell count increased, the LVEF increased, while the patient went from 1st No ART to 2nd on any combination of ART.

The prevalence of a particular CD4 cell count and LVEF relationships out of the total number of cases that were on any combination of ART or on no ART from one visit to another, was investigated (see figure 5.9).

The percentage of cases that were on no ART from one follow-up to another (total number of cases 48 - dark blue line on figure 5.9) shows that the highest percentage of cases were in the group in which the CD4 cell count decreased and the LVEF increased (33.33%). 27.08% of cases were in the group where the CD4 cell count and the LVEF increased; but 25% of cases were in the group in which the CD4 cell count decreased and the LVEF increased. The cases in the group in which the CD4 cell count increased and the LVEF decreased were only 14.58%.

The percentage of cases that were at first on no ART, but then went onto ART before the next follow-up (total number of 48 cases – orange line on figure 5.9) shows that the highest percentage of cases were in the group in which the CD4 cell count and the LVEF increased (54.17%). In the groups in which the CD4 cell count and the LVEF decreased, and in which the CD4 cell count decreased while the LVEF increased, the percentage of cases were very low (8.33% and 10.42% respectively). There were two groups in which the percentage of cases were 0%. One was when the CD4 cell count stayed constant while the LVEF increased, and the other one was when the CD4 cell count decreased and the LVEF stayed constant.

The percentage of cases that were at first on ART, but stopped the treatment before the next visit (total number of two cases – plum

coloured line on figure 5.9) shows only one very high peak of 100% in the group in which the CD4 cell count and the LVEF decreased. Please note that there were only two cases. Therefore, the very high percentage of 100%.

The percentage of cases which was on any combination of ART at both follow-up visits (total number of cases 80 – green line on figure 5.9) shows that the highest percentage of cases were in the group in which the CD4 cell count and the LVEF increased (36.25%), with a second high point in the group in which the CD4 cell count increased and the LVEF decreased. In all the other groups in which the CD4 cell count decreased (CD4 cell count and LVEF decreased, CD4 cell count decreased and LVEF increased and CD4 cell count decreased and LVEF stayed constant) the percentage of cases was very low (13.75%, 11.25% and 3.75% respectively). The lowest point occured when the CD4 cell count stayed constant and the LVEF increased (1.25%).

5.7. Influence of ART on the viral load and the LVEF.

Out of the 91 patients who had viral load studies and LVEF studies to compare to each other, 21 patients had 2 or more viral load studies and LVEF studies. This resulted in a total of 128 cases in which LVEF studies and viral load studies could be compared (see Figure 5.1). In comparing the cases with one another, the cases to be compared were named the 1st case and then the 2nd case. This means that, for example, where patient no. 1 had four cases of viral load and LVEF to compare, the comparison would be done like this:

30 January 2001	Viral load = >750000,	LVEF =50%, No ART	*1st No ART, **2nd No AR
20 March 2001	Viral load = >750000,	LVEF = 60%, No ART	*1st No ART, **2nd Any combination ART
30 July 2001	Viral load = 50000,	LVEF = 65%, ART	*1st Any combination ART, **2nd Any combination A
30 September 2001	Viral load = <400,	LVEF = 68%, ART	

** 1st means the patient was at the first set of variables that was compared to a 2nd set of variables on No ART/Any combination of ART.*
*** 2nd means the patient was at the second set of variables that was compared to the first set of variables on No ART/Any combination of ART*

Table 5.4: Summary of the influence of ART on the viral load and the LVEF.

		Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	
		Viral load↑ LVEF↑	**Viral load↑ LVEF↓**	**Viral load↓ LVEF↓**	**Viral load↓ LVEF↑**	**Viral load(c) LVEF↑**	**Viral load(c) LVEF↓**	**Viral load↑ LVEF(c)**	***Total***
		Frequency Row % Column%	**Frequency Row % Column%**	**Frequency Row % Column%**	**Frequency Row % Column%**	**Frequency Row % Column%**	**Frequency Row % Column%**	**Frequency Row % Column%**	
Row 1	***1st No ART **2nd No ART**	**5** 16.67 33.33	**7** 23.33 36.84	**3** 10 15	**10** 33.33 23.81	**3** 10 17.65 *(In all the cases the viral load stayed constant >750 000 RNA copies/ml)*	**2** 6.67 15.38 *(In all the cases the viral load stayed constant >750 000 RNA copies/ml)*	**0** 0 0	**30**
Row 2	**1st No ART 2nd Any combination of ART**	**3** 9.09 20	**2** 6.06 10.53	**6** 18.18 30	**21** 63.64 50	**1** 3.03 5.88 *(In all the cases the viral load stayed constant >750 000 RNA copies/ml)*	**0** 0 0	**0** 0 0	**33**
Row 3	**1st Any combination of ART 2nd No ART**	**0** 0 0	**1** 100 5.26	**0** 0 0	**0** 0 0	**0** 0 0	**0** 0 0	**0** 0 0	**1**
Row 4	**1st Any combination of ART 2nd Any combination of ART**	**7** 10.94 46.67	**9** 14.06 47.37	**11** 17.19 55	**11** 17.19 26.19	**13** 20.31 76.47 *(In all the cases the viral load stayed constant <400 RNA copies/ml)*	**11** 17.19 84.61 *(In 10 out of the 11 cases the viral load stayed constant <400 RNA copies/ml; in the other 1 case it stayed constant >750 000 RNA copies/ml)*	**2** 3.13 100	**64**
	Total	**15**	**19**	**20**	**42**	**17**	**13**	**2**	**128**

↑ Indicates an increase; ↓ Indicates a decrease; (c) indicates a variable that stayed constant

LVEF: Left ventricle ejection fraction (Normal value:>52% - 75%)

ART: Antiretroviral therapy

** 1st means the patient was at the first set of variables that was compared to a 2nd set of variables on No ART/Any combination of ART.*

*** 2nd means the patient was at the second set of variables that was compared to the first set of variables on No ART/Any combination of ART*

/ Ex: On 13 January 2003 the patient's viral load was 15 and the LVEF was 52% and at this stage the patient was on no ART; on 13 June 2003 the patient came for a follow-up. His viral load was 150 and the LVEF was 60% and the patient was at this stage on any combination of ART. Then the viral load increased, the LVEF increased, while the patient went from 1st No ART to 2nd on any combination of ART.*

This second part of the secondary objective focused on the influence that ART (antiretroviral treatment) had on the viral load and the LVEF (both normal and below normal values). With reference to flowchart 5.1, 209 patients had more than one LVEF and viral load that could

be compared to one another. To accommodate this situation in which one patient could have more than one follow-up visit to compare, we referred to each follow-up that was compared as a case. Therefore, one patient could have multiple cases that were compared. 101 patients had only one LVEF or only one CD4 cell count or viral load study. Out of the 91 patients who had viral load studies and LVEF studies that could be compared, 21 patients had more than two viral load studies and LVEF studies. To compare the viral load studies and the LVEF studies, each viral load and LVEF study that could be compared to each other was referred to as a case. There were 128 cases in which the viral load studies and LVEF studies could be compared.

During this discussion when, "1st and 2nd" are referred to, it means that one patient came for more than one follow-up. These follow-ups were compared to each other to see if the viral load and LVEF increased, decreased or stayed constant from one visit to another, as well as to see whether the patient was on any combination of ART or on no ART from one follow-up to another. Therefore, "1st" referred to the first set of variables that was compared to the following set of variables (at the second follow-up), which was named "2nd".

These results will be presented in two figures, figure 5.10 and figure 5.11. In figure 5.10 the column percentages from table 5.4 were

compared to each other. This means that the percentage of cases that were on any combination of ART or on no ART (rows in table 5.4) from one visit to another was compared to different relationships between the viral load and the LVEF (columns in table 5.4). This was done to see what the influence was of ART on the total number of cases in which there was a specific relationship between the viral load and the LVEF. Figure 5.11, on the other hand, shows the horizontal row percentages from table 5.4 which were compared to one another. This means that the percentage of cases that were on any combination of ART or on no ART (rows in table 5.4) from one visit to another was compared to different relationships between the viral load and the LVEF (columns in table 5.4). This was done to see what the percentage of cases with certain relationships between the viral load and the LVEF was when the cases were on any combination of ART or on no ART from one visit to another.

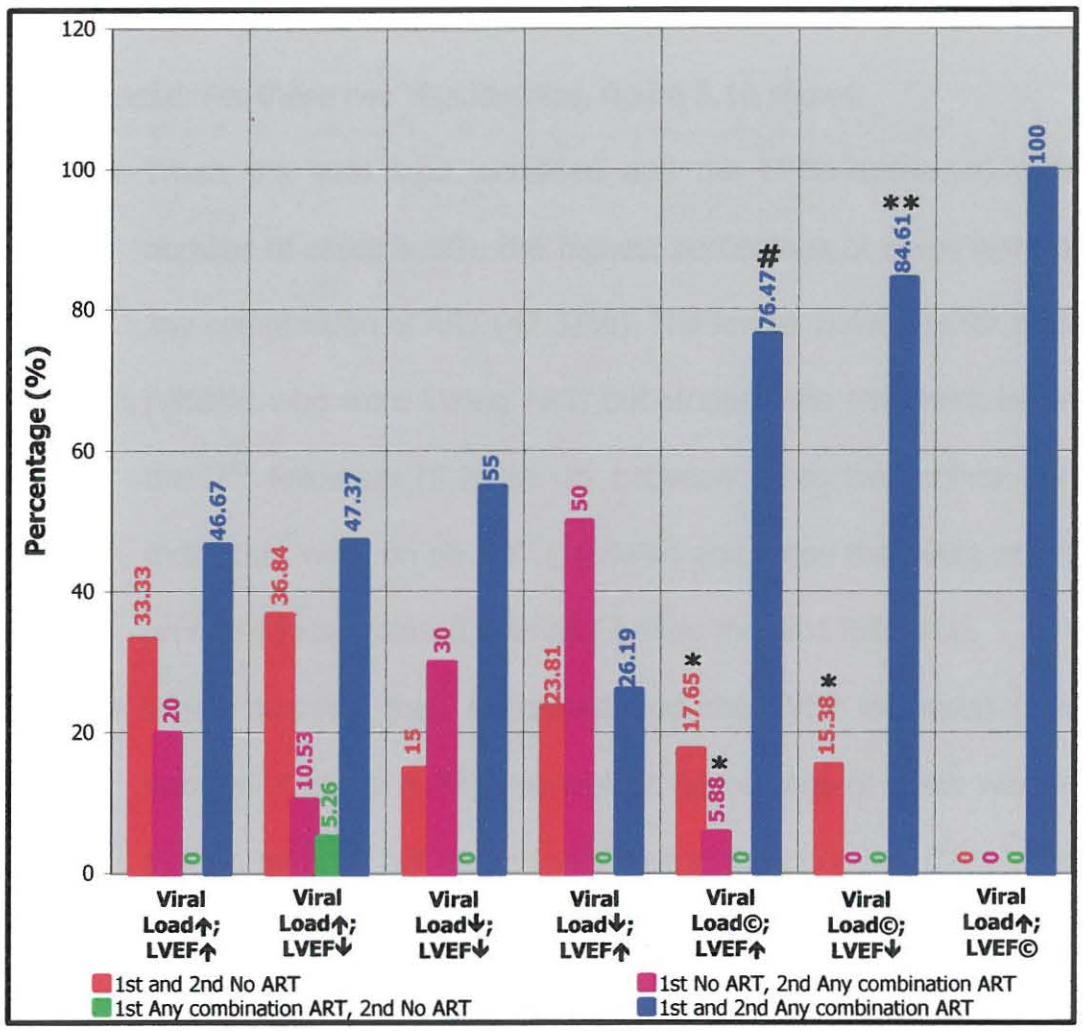

Figure 5.10: *Summary of the influence of ART on the viral load and LVEF from one visit to another (Column percentages – see Table 5.4).*

© Indicates a variable that stayed constant
↑Indicates an increase; ↓Indicates a decrease.
**: Viral load stayed constant ≥750 000 RNA copies/ml in all the cases.*
#: Viral load stayed constant ≤400 RNA copies/ml in all the cases.
***: Viral load stayed constant ≥750 000 RNA copies/ml in 7.69% of cases, and ≤400 RNA copies/ml in 76.92% of cases.*
LVEF: Left ventricle ejection fraction (Normal value:>52% - 75%)
ART: Antiretroviral therapy
VL: Viral load (measured in RNA copies/ml)
1st means the patient was at the first set of variables that was compared to a 2nd set of variables on No ART/Any combination of ART.
2nd means the patient was at the second set of variables that was compared to the first set of variables on No ART/Any combination of ART
Ex: On 13 January 2003 the patient's viral load was 15 and the LVEF was 52% and at this stage the patient was on no ART; on 13 June 2003 the patient came for a follow-up. His viral load was 150 and the LVEF was 60% and the patient was at this stage on any combination of ART. Then the viral load increased, the LVEF increased, while the patient went from 1st No ART to 2nd on any combination of ART.

In figure 5.10 the indirect proportional relationship between the viral load and the LVEF was two fold, namely when the viral load increased and the

LVEF decreased, or when the viral load decreased and the LVEF increased. For these two relationships, figure 5.10 shows:

- ⇨ When the viral load increased and the LVEF decreased (total number of cases = 19), the highest percentage of cases were on any combination of ART (47.37%). The lowest point was for those patients who were taking ART, but stopped the treatment before the 2nd follow-up (5.26%). In between these two points were those that were on no ART (36.84%) and those that were at first on no ART, but started with ART before the next follow-up.
- ⇨ When the viral load decreased and the LVEF increased (total number of cases = 42), the highest percentage of cases were at first on no ART, but started ART before the next visit (50%). The second highest point was for those cases that were on any combination of ART during both visits (26.19%). Those cases that were on no ART were 23.81%. There were no cases (0%) that were on ART at first and then stopped the treatment.

The direct proportional relationship between the viral load and the LVEF from figure 5.10 shows two possibilities. First, the viral load and the LVEF increased, and secondly, the viral load and the LVEF decreased. For these two relationships figure 5.10 shows:

- ⇨ That when the viral load and the LVEF increased (total of 15 cases), the highest percentage of cases were on any combination of ART (46.67%). The second highest point was for those cases

that were on no ART (33.33%). 20% of cases were at first on no ART, but started with ART before the next visit. There were no cases (0%) in which the patients were at first on ART, but stopped the treatment before the next visit.

- ⇨ That when the viral load and LVEF decreased (total of 20 cases), the highest point was for those cases that were on ART during both visits (55%). The second highest point was for those cases that went onto ART before the second follow-up (30%). Only 15% of cases were on no ART. There were no (0%) cases in which the patients were first on ART and then stopped taking it before the next visit.

The relationships between the viral load and the LVEF where one of the variables stayed constant were three different relationships. First, the viral load stayed constant and the LVEF increased, secondly the viral load stayed constant and the LVEF decreased, and thirdly the viral load increased and the LVEF stayed constant. For these three relationships figure 5.10 shows that:

- ⇨ When the viral load stayed constant and the LVEF increased (total number of cases = 17), the viral load of 14 cases stayed constant below 400 RNA copies/ml, and the viral load of three cases stayed constant above 750 000 RNA copies/ml. The highest percentage of cases were on any combination of ART during both follow-up visits (76.47%). In all of these 76.47% of cases, the viral load

stayed constant below 400 RNA copies/ml. The second highest point occurred in those cases that were on no ART (17.65%). In all of these 17.65% of cases, the viral load stayed constant above 750 000 RNA copies/ml. 5.88% of cases went from no ART onto ART before the next follow-up. The viral load of these percentage of cases stayed constant above 750 000 RNA copies/ml. Out of these findings one could say that the highest point was when the the viral load stayed constant below 400 RNA copies/ml, while the LVEF increased, and they were on ART from one visit to another. This shows a very positive influence of the ART on the viral load, because the goal is to keep the viral load below 400 RNA copies/ml for as long as possible.

⇨ When the viral load stayed constant and the LVEF decreased (total number of cases = 13). Out of these thirteen cases, the viral load of three cases stayed constant above 750 000 RNA copies/ml and the viral load of ten cases stayed constant below 400 RNA copies/ml. The highest percentage of cases were on any combination of ART from one follow-up to the next (84.61%). From this 84.61% of cases the viral load of 76.92% cases was below 400 RNA copies/ml, and the viral load of 7.69% cases was above 750 000 RNA copies/ml. The second highest percentages of cases were on no ART (15.38%). All of the viral loads of these 15.38% cases were above 750 000 RNA copies/ml. There were no (0%) cases that went onto ART (that was at first on no ART and

then went onto ART before the next visit), or that were on ART and then stopped the treatment before the next visit

⇨ When the viral load increased and the LVEF stayed constant (total number of cases = 2). This was an indeterminate relationship, because there was no definite relationship between these two cases and the LVEF did not stay constant at the same value in the two cases. Both of these cases were on ART from one visit to another. Therefore the very high percentage of 100%.

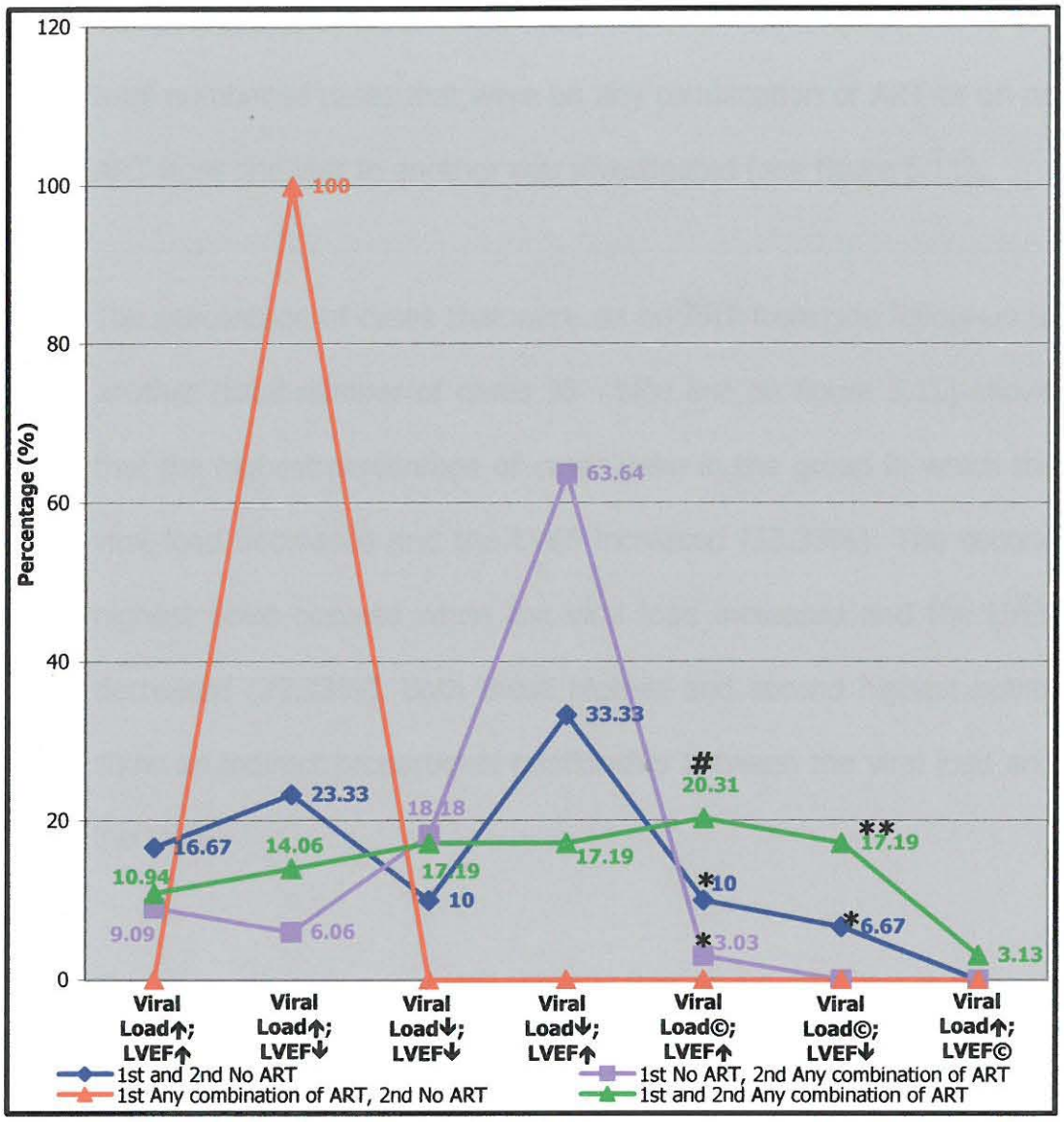

Figure 5.11: *Summary of the influence of ART on the viral load and the LVEF from one visit to another (Row percentages – see Table 5.4).*

© Indicates a variable that stayed constant
↑Indicates an increase; ↓Indicates a decrease.
**: Viral load stayed constant ≥750 000 RNA copies/ml in all the cases.*
#: Viral load stayed constant ≤400 RNA copies/ml in all the cases.
***: Viral load stayed constant ≥750 000 RNA copies/ml in 1.56% of cases, and ≤400 RNA copies/ml in 15.63% of cases.*
LVEF: Left ventricle ejection fraction (Normal value:>52% - 75%)
ART: Antiretroviral therapy
VL: Viral load (measured in RNA copies/ml)
1st means the patient was at the first set of variables that was compared to a 2nd set of variables on No ART/Any combination of ART.
2nd means the patient was at the second set of variables that was compared to the first set of variables on No ART/Any combination of ART
Ex: On 13 January 2003 the patient's viral load was 15 and the LVEF was 52% and at this stage the patient was on no ART; on 13 June 2003 the patient came for a follow-up. His viral load was 150 and the LVEF was 60% and the patient was at this stage on any combination of ART. Then the viral load increased, the LVEF increased, while the patient went from 1st No ART to 2nd on any combination of ART.

The prevalence of certain viral load and LVEF relationships out of the total number of cases that were on any combination of ART or on no ART from one visit to another was investigated (see figure 5.11).

The percentage of cases that were on no ART from one follow-up to another (total number of cases 30 - blue line on figure 5.11) shows that the highest percentage of cases were in the group in which the viral load decreased and the LVEF increased (33.33%). The second highest point occured when the viral load increased and the LVEF decreased (23.33%). Both these highest and second highest points show an indirect proportional relationship between the viral load and the LVEF.

The percentage of cases that were at first on no ART, but then went onto ART before the next follow-up (total number of 33 cases – purple line on figure 5.11) shows that the highest percentage of cases were in the group where the viral load decreased and the LVEF increased (63.64%). The second highest point occured in the group where the viral load and the LVEF decreased (18.18%).

The percentage of cases that were at first on ART, but stopped the treatment before the next visit (total number of one case – orange line on figure 5.11) shows only one very high peak of 100% in the group in which the viral load increased and the LVEF decreased.

Please note that there was only one case, therefore, the very high percentage of 100%.

The percentage of cases at both follow-up visits on any combination of ART (total number of cases 64 – green line on figure 5.11) shows that the highest percentage of cases were in the group in which the viral load stayed constant and the LVEF increased (20.31%). The viral load of all of these 20.31% of cases stayed constant below 400 RNA copies/ml. The second highest points were in the groups in which the viral load decreased and the LVEF increased, the viral load and LVEF decreased, and the viral load stayed constant (the viral load of 10 out of the 11 cases, i.e. 15.63% of the 17.19% of cases, stayed constant below 400 RNA copies/ml) while the LVEF decreased, all at 17.19%

5.8. Percentage of cases on ART versus those on no ART at certain CD4 cell count cut-off points and with LVEF below normal (≤52%).

Out of the population there were 114 cases of patients with CD4 cell counts and LVEF studies in which the LVEF study was below normal (≤52%). Out of these cases 67 were not on ART and 47 were on any combination of ART (see figure 5.1 and Appendix A, Table A.3, Part 1).

The cut-off points for the CD4 cell count were 50, 100, 150, 200, 250 300, 350, >350 cells/ml. The >350 cells/ml cut-off point (i.e. 0 - >350) includes all the cases defined above (see Appendix A, Table A.3, Part 1 – 8).

Here the focus was on comparing the percentage of cases with below normal LVEF on any combination of ART to those that were on no ART when their LVEF was below normal, at particular CD4 cell count cut-off points. Out of the 310 patients who had CD4 cell counts and LVEF studies, irrespective of the amount of cases per patient, 91 patients had an LVEF of below normal (≤52%). Sixteen of these patients had more than one case per patient. This resulted in 114 cases to be investigated (see figure 5.1). Out of these 114 cases, 67 cases were not on ART and 47 cases were on any combination of ART (see Appendix A, Table A.3, Part 1).

The cut-off points for the CD4 cell counts were 50, 100, 150, 200, 250, 300, 350, >350 cells/ml. The >350 cells/ml cut-off point (i.e. 0 - >350) includes all the cases defined above (see Appendix A, Table A.3, Part 1 – 8).

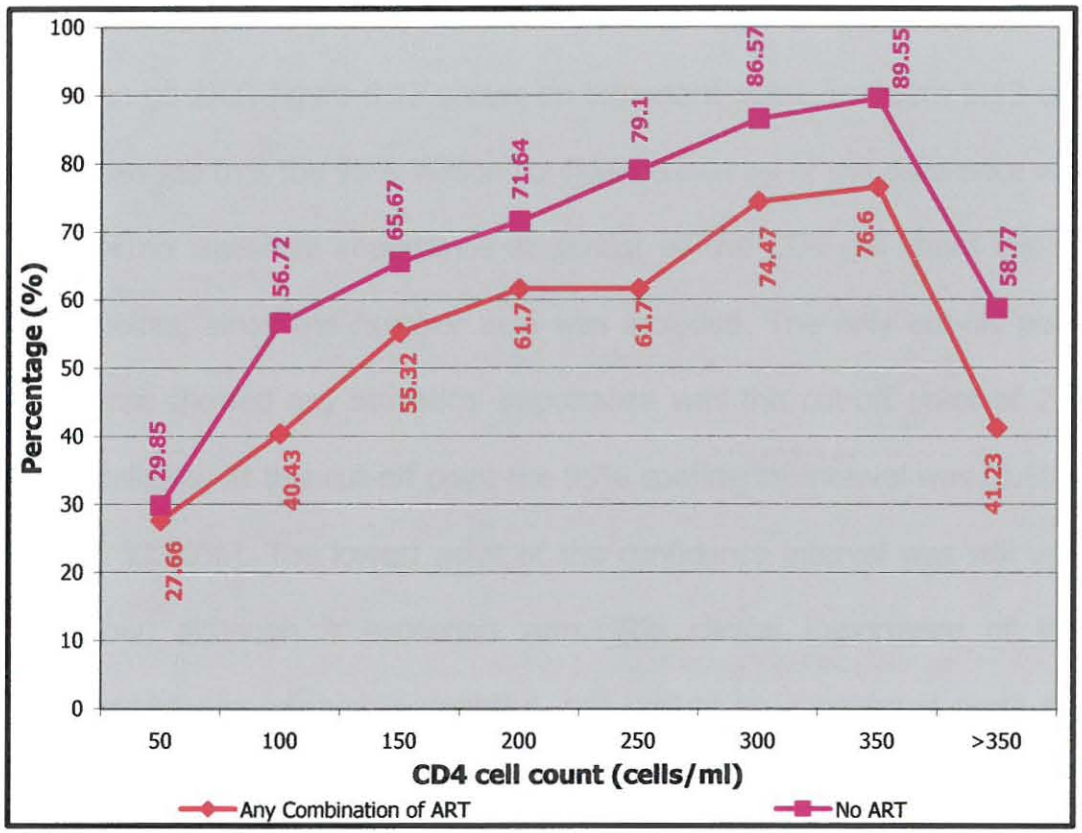

Figure: 5.12: *Percentage of cases on ART versus those not on ART at certain CD4 cell count cut-off points and with LVEF below normal (≤52%).*
LVEF: Left ventricle ejection fraction (Normal value:>52% - 75%)
ART: Antiretroviral therapy.

Figure 5.12 shows that the percentage of cases whose LVEF was below normal and that were not on any ART was more than those who were on ART. The percentages of cases in both groups of cases (those who were on ART with LVEF below normal, and those who were on no ART with LVEF below normal) increased as the CD4 cell

count increased up to the cut-off point of 350 cells/ml, and then both decreased steeply when the CD4 cell count reached the >350 cells/ml cut-off point.

Even though figure 5.12 shows that the occurrence of a below normal LVEF was less in those cases that were on ART than those that were on no ART, figure 5.13 shows an important point. In figure 5.13 one can see that the 95% Wilson confidence interval of the difference was of no statistical importance at almost all the CD4 cell count cut-off points, since the number zero was included. The only cut-off point that showed any statistical importance was the cut-off point of 250 cells/ml. At this cut-off point the 95% confidence interval was [0.60% ; 33.80%]. The lowest point of the confidence interval was still very low, although it exceeded zero. The clinical importance of this confidence interval is doubtful, but should be considered since the upper limit of the 95% confidence interval is high.

(See Appendix A, Table A.3, Part 1 – 8).

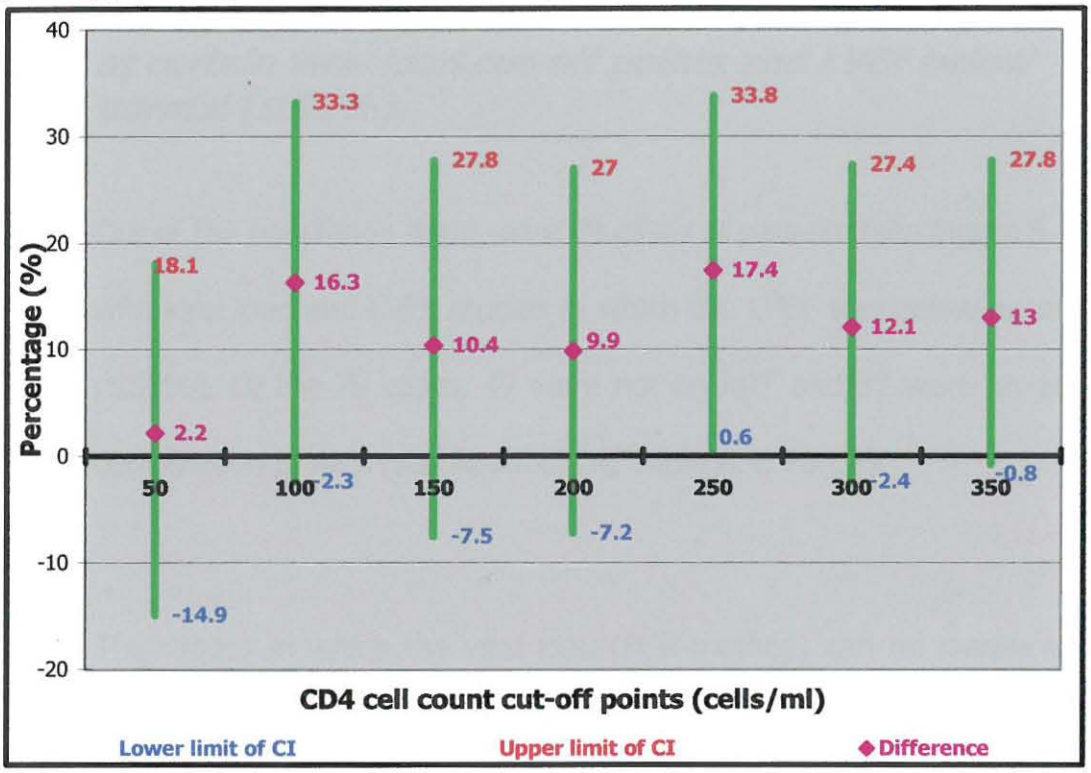

Figure 5.13: *Graphic presentation of the 95% Wilson confidence interval of the percentage difference between those on ART versus those on no ART, when the CD4 cell count was at specific cut-off points and the LVEF below normal (≤52%).*

LVEF: Left ventricle ejection fraction (Normal value:>52% - 75%)
CI: 95% Wilson confidence interval of the percentage difference.
ART: Antiretroviral therapy
The difference in the percentages was calculated by the difference between the percentage of cases with below normal LVEF in cases with CD4 cell count less or equal to (≤) the cut-off point of those cases that were on no ART and those cases that were on any combination of ART.

5.9. Percentage of cases on ART versus those on no ART at certain viral load cut-off points and LVEF below normal (≤52%).

Out of the population there were 79 cases of patients (see Figure 5.1) with viral load and LVEF studies in which the LVEF was below normal (≤52%). Of the 79 cases, 47 were not on ART and 32 were on any combination of ART (see Appendix A, Table A.4, Part 1).

The ranges in which the viral load (PCR-method) can be measured, are <400 RNA copies/ml (in some cases when the doctor requested the ultra HIV PCR viral load, then the lowest limit is <50 RNA copies/ml) up to >750 000 RNA copies/ml. The cut-off points in this study were ≤1 000 (i.e. $\leq 10^3$), 10 000 (i.e. 10^4), 100 000 (i.e. 10^5) and 1 000 000 (i.e. 10^6). Therefore the lowest value of viral load (< 400 RNA copies/ml or in some cases <50 RNA copies/ml) were included in the ≤1 000 cut-off point, and the highest value of viral load (>750 000 RNA copies/ml) was included in the cut-off point of <1 000 000. The cut-off point of <1 000 000 would therefore include all the above defined cases (i.e. 0 – 1 000 000) (see Appendix A, Table A.4, Part 1 – 4).

Here the focus was on comparing the percentage of cases with below normal LVEF on any combination of ART to those who were not on

ART when their LVEF was below normal, at certain viral load cut-off points. Out of the 310 patients who had viral load and LVEF studies, irrespective of the number of cases per patient, 66 patients had an LVEF of below normal (≤52%). Ten of these patients had more than one case per patient. This resulted in 79 cases to be investigated (see figure 5.1). Out of these 79 cases, 47 cases were not on ART and 32 on any combination of ART (see Appendix A, Table A.4, Part 1 – 4).

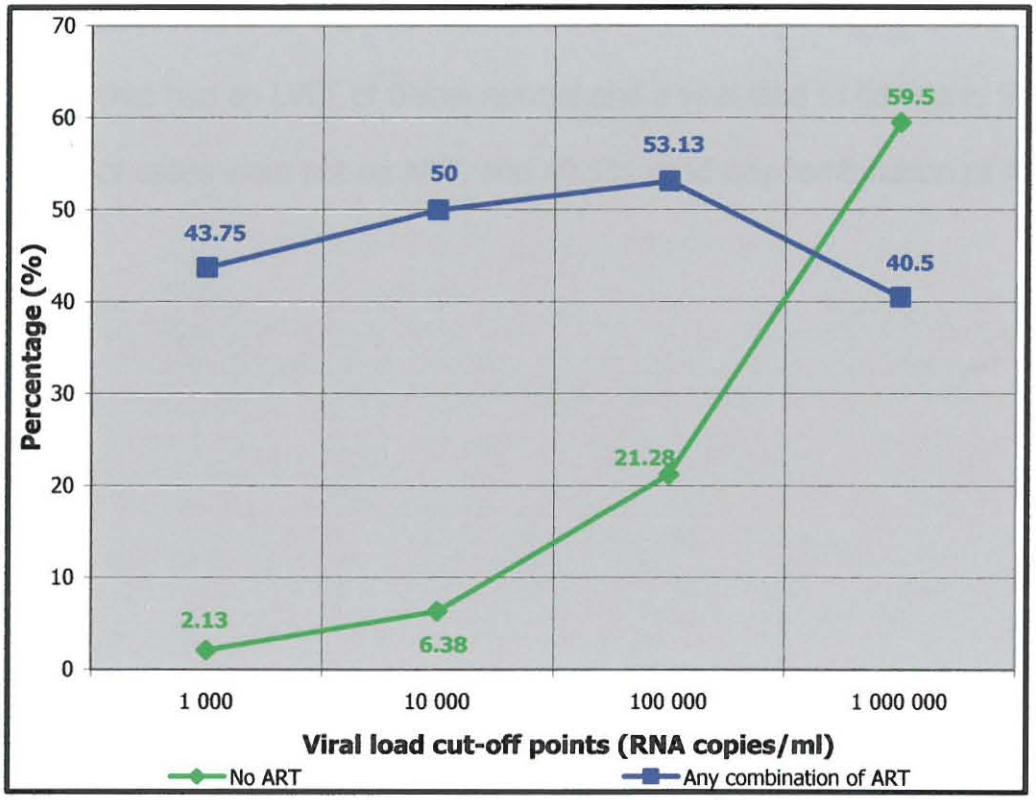

Figure 5.14: *Percentage of cases on any combination of ART versus those who were on no ART, at certain viral load cut-off points in which the LVEF was below normal (≤52%).*

LVEF: Left ventricle ejection fraction (Normal value:>52% - 75%)
ART: Antiretroviral therapy

Figure 5.14 shows that the percentage of cases with below normal LVEF on ART occurred more frequently than those with below normal LVEF that were on no ART, up to the cut-off point of 100 000 RNA

copies/ml. When the viral load was >100 000 RNA copies/ml, there was a sudden decrease in the percentage of cases with below normal LVEF on ART, to a point that was below those cases who were on no ART. There was also a steady increase in the percentage of cases with below normal LVEF on no ART, after the cut-off point of 100 000 RNA copies/ml. This might be due to the fact that when the viral load is very high (above 100 000 RNA copies/ml) the patients are usually not on ART or they have stopped taking the ART. Out of all 79 cases that had an LVEF of below normal and a viral load to compare, 59.5% of cases were not on ART, and 40.5% used any combination of ART.

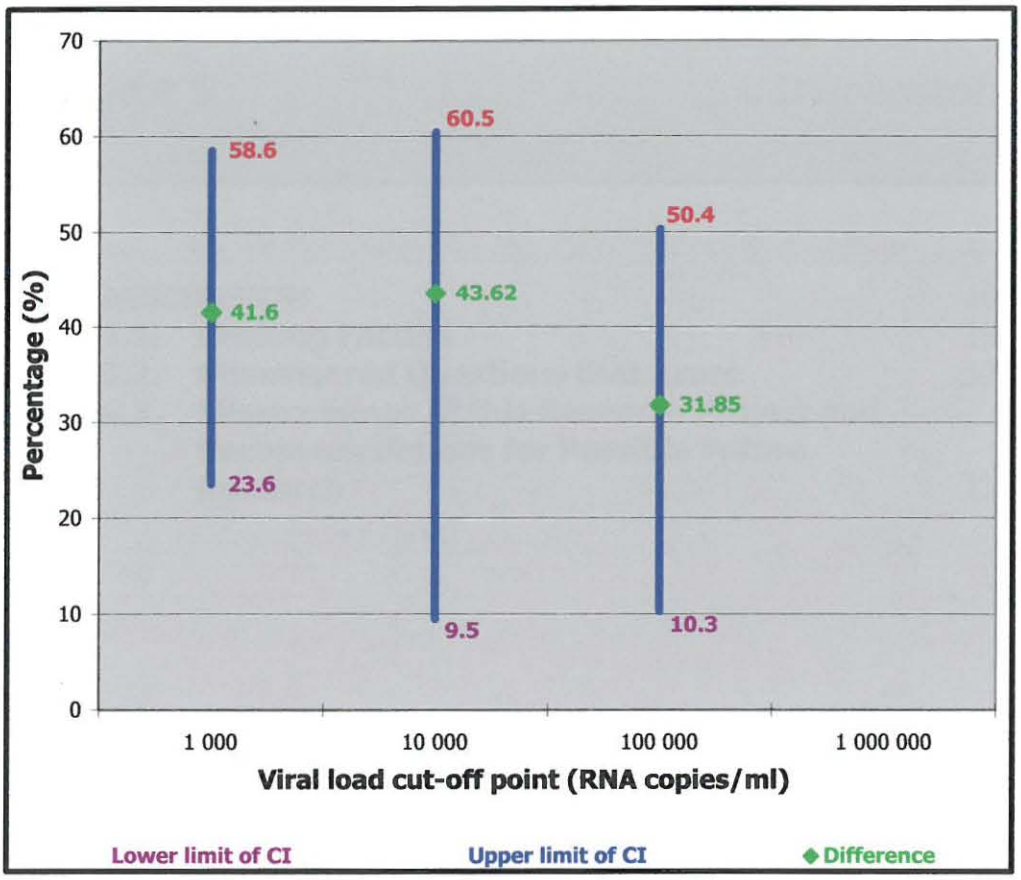

Figure 5.15: *Graphic presentation of the 95% Wilson confidence interval for the percentage difference of cases on no ART versus those on any combination of ART at certain viral load cut-off points, in which the LVEF was below normal (≤52%).*

LVEF: Left ventricle ejection fraction (Normal value:>52% - 75%)
CI: 95% Wilson confidence interval for the percentage difference
ART: Antiretroviral therapy
The difference in the percentage was calculated by the difference between the percentage of cases with below normal LVEF in cases with viral load smaller or equal (≤) to the cut-off point for the cases that were on any combination of ART and for the cases that were on no ART.

The 95% Wilson confidence interval for the percentage difference for this part of the secondary objective is shown in figure 5.15. All of the 95% confidence intervals were of statistical importance, since the number zero was not included in any one of the confidence intervals. The highest confidence interval [9.50% ; 60.50%] occurred at the cut-off point of 10 000 RNA copies/ml.

Chapter 6 Discussion

A total of 458 patients were included in the research project after the exclusion and inclusion criteria had been met. 138 patients did not have an LVEF study for reasons discussed under 6.1. They were excluded. Ten patients did not have any CD4 cell count or viral load tests done, usually for financial reasons, or patients passed away before these tests could be done, or did not come back for follow-up visits after the initial consultation (see figure 5.1). There were 310 patients left. In this group of patients 165 were female and 145 were male, with ages ranging between 20 – 70 years (average age was 45 years).

In this research study it was shown that there is a direct proportional relationship between the CD4 cell count and the LVEF (see figure 5.2), and an indirect proportional relationship between the viral load and the LVEF (see figure 5.3). The below normal LVEF (≤52%) occurred most frequently when the CD4 cell count was below 100 cells/ml (see figure 5.4) – which correlates well with the findings of Currie *et al.* (1998) – and/or the viral load was above 100 000 RNA copies/ml (see figure 5.5), i.e. a very advanced stage of HIV infection. Schlant and Alexander (1994) also indicated that echocardiographic evidence of left ventricle dysfunction is more common in patients who are the furthest along in the course of HIV disease. Lipshultz *et al.* (1998) reported that the degree of depression of LVEF correlates with the extent of immune dysfunction at baseline

but not in the long term, suggesting that the CD4 cell count may not be a useful surrogate marker of HIV-associated left ventricular dysfunction.

Figure 5.4 shows that there was a steady decline in the percentage of cases with LVEF below normal as the CD4 cell count increased, but there was an unexpected fall in the prevalence of the LVEF being below normal (≤52%) when the CD4 cell count was between 0 – 50 cells/ml. Here the percentage of cases was 35.87% as opposed to the 39.86% when the CD4 cell count was at the cut-off point of 100 cells/ml. The difference in the percentage of cases with below normal LVEF between cases with ≤100 cells/ml and ≤50 cells/ml, was 3.99%. No clear reason could be found as to why this occurred. This prevalence is still unanswered. Ince *et al.* (1999) also found that the greatest incidence of dilated cardiomyopathy was in those with depressed CD4 cell counts in particularly in those with CD4 cell counts of <300 cells/ml.

In figure 5.8 the direct proportional relationship between the CD4 cell count and the LVEF was investigated. Here there were 2 different relationships between the CD4 cell count and the LVEF, namely the CD4 cell count increased and the LVEF increased, and secondly the CD4 cell count decreased and the LVEF decreased. For these two relationships figure 5.8 shows the following:

- ⇨ When the CD4 cell count and the LVEF increased, the highest percentage of cases out of this group (total of 68 cases) were on any combination of ART (42.65%), while the second highest percentage of cases occured when the patients went from no ART onto some or other combination of ART (38.24%). The percentage of cases that were on no ART came to only 19.11% of the patients whose CD4 cell count and LVEF increased.
- ⇨ When the CD4 cell count and the LVEF decreased, the highest percentage of cases out of this group (total of 29 cases) were on no ART (41.38%). The second highest point occured in those patients who were on any combination of ART (37.93%). Those cases who were at first on no ART and then went onto ART, came to 13.79%, while those cases who were at first on some sort of combination of ART and then stopped it (i.e. no ART) amounted to only 6.90%.

The indirect proportional relationship between the CD4 cell count and the LVEF in figure 5.8 shows two possible relationships, namely the CD4 cell count increased, while the LVEF decreased, and CD4 cell count decreased while the LVEF increased. The following can be seen from figure 5.8:

- ⇨ When the CD4 cell count increased and the LVEF decreased (total of 47 cases), the highest percentage of cases were

on any combination of ART (57.45%), while the second highest point occured in those cases that were at first on no ART, but went onto ART before the second follow-up visit (27.66%). The two lowest points occurred in those cases that were not on ART (14.89%) and those that were at first on ART, but then stopped it before the second follow-up visit (0%).

- ⇨ When the CD4 cell count decreased and the LVEF increased (total of 30 cases), the highest percentage of cases occurred when they were on no ART from one visit to another (53.33%), with those that were on any combination of ART on both visits, second (30%). The two lowest points occurred in those that were on no ART at first, but began with ART before the next visit (16.67%), and the lowest point occurred in those that were on ART at first, but stopped the ART. This amounted to 0%.

In the indeterminate relationship between the CD4 cell count and the LVEF one of the variables stayed constant. Figure 5.8 shows the following:

- ⇨ When the CD4 cell count stayed constant and the LVEF increased (total of one case), the percentage of cases that were on ART with both visits, was 100%. It is important to note that in this relationship between the CD4 cell count

and the LVEF, there was only one case, therefore the very high peak of 100%.

- ⇨ When the CD4 cell count decreased, while the LVEF stayed constant (total of three cases), the percentage of cases that were on any combination of ART was the highest, with 100%. It is important to note that in this relationship between the CD4 cell count and LVEF, there were only three cases and all three cases were on ART, therefore the very high peak of 100%.

In summarizing all the findings from figure 5.8, one can see that in all the cases in which the CD4 cell count increased, the highest percentage of cases were on any combination of ART. When the CD4 cell count decreased, the highest percentage of cases were on no ART, with exception of those three cases in the group in which the CD4 cell count decreased, while the LVEF stayed constant. In the one case when the CD4 cell count stayed constant, the highest percentage of cases were on any combination of ART during both visits. In contrast to this, when the LVEF increased, the highest percentage of cases were sometimes on any combination of ART (in the group in which the CD4 cell count and the LVEF increased, and in the one case in which the CD4 cell count stayed constant and the LVEF increased) and sometimes the highest percentage of cases were on no ART (in the group in which the CD4 cell count decreased and

the LVEF increased). When the LVEF decreased, the highest percentage of cases were sometimes those cases that were on any combination of ART during both visits (in the group in which the CD4 cell count increased and the LVEF decreased), and sometimes those cases that were on no ART (in the group in which the CD4 cell count decreased and the LVEF decreased). Therefore it seems as if the ART had a positive effect on the CD4 cell count in such a way that when the patients were on ART, the CD4 cell count increased, and when the patients were on no ART, the CD4 cell count tended to decrease. But no relationship between the LVEF and ART could be shown, i.e. one could not say that ART caused the LVEF to increase or decrease. This brings us back to figure 5.2, which indicates that the CD4 cell count and the LVEF had a direct proportional relationship. Therefore, one could assume that if the ART had a positive effect on the CD4 cell count (i.e. the CD4 cell count increased when the patients were on ART), the CD4 cell count that increased would have an increasing effect on the LVEF, although the ART did not have a direct effect on the LVEF.

In summarizing the findings from figure 5.9, one can see that in all the groups in which the CD4 cell count increased, the highest percentage of cases were on ART or went onto ART (no ART at first, but before the 2nd follow-up, they started with ART). In the groups in which the CD4 cell count decreased, the highest percentage of cases

were on no ART. Therefore it seemed as if the ART had a positive effect (to increase the CD4 cell count) on the CD4 cell counts. On the other hand, in the groups in which the LVEF increased, some of the highest percentage of cases were on ART, some went onto ART, and others were on no ART. The same was seen when the LVEF decreased. Some of the highest percentage of cases were on ART, while other high percentages were on no ART. Therefore it did not seem as if the ART resulted in the LVEF increasing or decreasing. From figure 5.2 it was seen that there was a direct proportional relationship between the CD4 cell count and the LVEF. Thus, if the ART had a positive effect on the CD4 cell count, and the CD4 cell count increased, then one could expect that the LVEF would also increase, rather than decrease.

The findings of figure 5.8 and 5.9 correlates with the findings of Pugliese *et al.* (2000) who said that HAART does dramatically decrease the incidence of cardiac involvement in patients with HIV infection as opposed to patients only treated with one NRTI.

In figure 5.10 the indirect proportional relationship between the viral load and the LVEF was twofold; namely when the viral load increased and the LVEF decreased; or when the viral load decreased and the LVEF increased. For these two relationships, figure 5.10 shows that:

- When the viral load increased and the LVEF decreased (total number of cases = 19), the highest percentage of cases were on any combination of ART (47.37%). The lowest point occurred when those that were taking ART stopped the treatment before the 2nd follow-up (5.26%). In between these two points were those who were on no ART (36.84%) and those who were at first on no ART, but started with ART before the next follow-up.
- When the viral load decreased and the LVEF increased (total number of cases = 42), the highest percentage of cases were at first on no ART, but started with ART before the next visit (50%). The second highest point occurred in those cases that were on any combination of ART during both visits (26.19%). 23.81% of cases were on no ART. There were no cases (0%) that were on ART at first and then stopped the treatment.

The direct proportional relationship between the viral load and the LVEF from figure 5.10 shows two possible relationships. First, the viral load and the LVEF increased, and secondly, the viral load and the LVEF decreased. For these two relationships figure 5.10 shows:

- That when the viral load and LVEF increased (total of 15 cases), the highest percentages of cases were on any combination of ART (46.67%). The second highest point was for those cases that were on no ART (33.33%). Those cases

that were at first on no ART, but started with ART before the next visit, amounted to 20%. There were no cases (0%) in which the patients were at first on ART, but stopped the treatment before the next visit.

- That when the viral load and LVEF decreased (total of 20 cases), the highest point occurred in those cases that were on ART during both visits (55%). The second highest point occurred in those cases that went onto ART before the second follow-up (30%). Only 15% were on no ART. There were no (0%) cases in which the patients were first on ART and then stopped taking it before the next visit.

There were three different relationships between the viral load and the LVEF where one of the variables stayed constant. First, the viral load stayed constant and the LVEF increased, secondly the viral load stayed constant and the LVEF decreased, and thirdly the viral load increased and the LVEF stayed constant. For these three relationships figure 5.10 shows that:

- When the viral load stayed constant and the LVEF increased (total number of cases = 17), the viral load of 14 cases stayed constant below 400 RNA copies/ml, and the viral load of three cases stayed constant above 750 000 RNA copies/ml. The highest percentage of cases were on any combination of ART during both follow-up visits (76.47%). In all of these 76.47% of cases, the viral load

stayed constant below 400 RNA copies/ml. The second highest point occurred in those cases that were on no ART (17.65%). In all of these 17.65% of cases, the viral load stayed constant above 750 000 RNA copies/ml. 5.88% of cases went from no ART onto ART before the next follow-up. The viral load of these percentage of cases stayed constant above 750 000 RNA copies/ml. Out of these findings one could say that the highest point was when the the viral load stayed constant below 400 RNA copies/ml, while the LVEF increased, and they were on ART from one visit to another. This shows a very positive influence of the ART on the viral load, because the goal is to keep the viral load below 400 RNA copies/ml for as long as possible.

⇨ When the viral load stayed constant and the LVEF decreased (total number of cases = 13). Out of these thirteen cases, the viral load of three cases stayed constant above 750 000 RNA copies/ml and the viral load of ten cases stayed constant below 400 RNA copies/ml. The highest percentage of cases were on any combination of ART from one follow-up to the next (84.61%). From this 84.61% of cases the viral load of 76.92% cases was below 400 RNA copies/ml, and the viral load of 7.69% cases was above 750 000 RNA copies/ml. The second highest percentages of cases were on no ART (15.38%). All of the viral loads of these 15.38% cases were above 750 000 RNA copies/ml. There were no (0%) cases that went onto ART (that was at first on no ART and

then went onto ART before the next visit), or that were on ART and then stopped the treatment before the next visit

⇨ When the viral load increased and the LVEF stayed constant (total number of cases = 2). This was an indeterminate relationship, because there was no definite relationship between these two cases and the LVEF did not stay constant at the same value in the two cases. Both of these cases were on ART from one visit to another. Therefore the very high percentage of 100%.

In summarizing all the findings from figure 5.10, one can see that when the viral load stayed constant below 400 RNA copies/ml, all cases were on any combination of ART, or went onto ART (at the first visit the patient was on no ART, but went onto ART before the next visit). In the cases in which the viral load stayed constant above 750 000 RNA copies/ml, none of the cases were on ART (no ART). This shows a very positive influence of ART on the viral load, since the goal is to get the viral load below 400 RNA copies/ml and keep it there for as long as possible. In all the cases in which the viral load decreased, the highest and second highest percentage of cases were all either on ART on both visits, or they went onto ART (at first they were on no ART, but went onto ART before the next visit). This also showed a positive influence of ART on the viral load. One should then expect that when the patients were on no ART, the viral load would have increased or stayed constant above 750 000 RNA copies/ml. There were two exceptions to this positive influence of ART on the

viral load. This was seen in the cases in which the viral load increased: the viral load increased, although the highest percentage of cases were on ART and the second highest percentage of cases were on no ART. One would have expected that the highest percentage of cases would not be ART. A possible explanation might be that the large number of patients that were on ART might have developed viral resistance, and therefore the viral load increased, even though the patients were on ART. Regarding the second highest point, the patients were on no ART, and their viral load increased. When the LVEF increased, decreased, or stayed constant, there were no direct influences of the ART on the LVEF. Figure 5.3 shows there is an indirect proportional relationship between the viral load and the LVEF. From figure 5.3 and figure 5.10 one could say that if the ART had a positive effect on the viral load (i.e. viral load decreased) then there is a large possibility that the viral load reduction would cause the LVEF to increase. Thus the ART had an influence on the viral load and the viral load had an influence on the LVEF. ART therefore has an indirect positive effect on the LVEF. These findings correlate with the findings of Barbaro (1998b). He found that the incidence of dilated cardiomyopathy was influenced more by the extent of immunodeficiency than by the type of antiretroviral therapy (Barbaro, 1998b).

In summarizing the findings from figure 5.11, one can see that at the highest points of cases that were on any combination of ART, the viral loads either decreased or stayed constant. In the relationships where the viral load stayed constant, the highest number of cases were on ART from one follow-up to another. In all of these cases, the viral load stayed constant below 400 RNA copies/ml, which is the goal viral load. In those cases that went onto ART (at first they were on no ART, but started ART before the next visit) the highest points also occurred when the viral load decreased. From this, one could say that the ART had a positive effect on the viral load. When the cases were on no ART, the highest point occurred when the viral load decreased and the LVEF increased. The reason for this is not clear. It might be due to life changes, etc., but this cannot be confirmed. If one looks at the second highest points of the cases that were on no ART, the viral load did increase. The one case in which the patient was first taking ART, but discontinued the treatment, the viral load increased.

On the other hand, there was no direct influence of the ART on the LVEF. One could only say, as was seen in figure 5.3, that the viral load and the LVEF had an indirect proportional relationship to each other in most cases and that the ART had a positive effect on the viral load in most cases. Therefore, if the ART improved the viral load (i.e. viral load decreases), then one would expect to see that the

LVEF would increase. Thus the ART did have a positive influence on the viral load and, via the viral load, a positive influence on the LVEF.

Barbaro (1998b) found that among patients who received zidovudine (from the NRTI group), the incidence of cardiomyopathy was greater in those with a CD4 cell count of less than 300 cell/ml, but this same study also mentioned that the difference observed in the incidence of dilated cardiomyopathy among the risk groups was influenced more by the extent of immunodeficiency than by the type of ART. Lipshultz (1998) also indicated that zidovudine (from the NRTI group) neither worsened nor ameliorated progressive cardiac changes in HIV infected patients. Millei *et al.* (1998) mentioned that myocardial dysfunction may also result indirectly via drugs such as zidovudine.

Figure 5.12 shows that a greater percentage of cases with a below normal LVEF (≤52%) were on no ART, compared with those who were on ART. The percentage of cases in both groups (those who were on ART and those who were on no ART) increased as the CD4 cell count increased up to the cut-off point of ≤350 cells/ml, and then steeply decreased when the CD4 cell count reached the >350 cells/ml cut-off point (see figure 5.13). It was also shown that the only cut-off point that had any statistical importance (according to the 95% Wilson confidence interval of the difference) was the cut-off point of 250 cells/ml. Even though the clinical importance of this confidence

interval [0.60% ; 33.80%] is doubtful, it should still be considered since the upper limit of the 95% confidence interval is high.

Figure 5.14 shows that a higher percentage of cases with below normal LVEF were on ART in comparison with those with below normal LVEF that were on no ART up to the cut-off point of 100 000 RNA copies/ml. When the viral load was >100 000 RNA copies/ml, there was a sudden decrease in the percentage of cases with a below normal LVEF who were on ART, to a point that was below those cases who were on no ART. There was also a steady increase in the percentage of cases with a below normal LVEF who were on no ART, after the cut-off point of 100 000 RNA copies/ml. There is no outstanding reason why this occurred, and further research of this specific aspect needs to be done. Of the 79 cases that had a below normal LVEF and a viral load to compare, 59.5% were on no ART, and 40.5% used any combination of ART.

Figure 5.6 up to figure 5.14 agree with the findings of Pugliese *et al.* (2000). Pugliese *et al.* (2000) said that HAART (highly active antiretroviral therapy) does dramatically decrease the incidence of cardiac involvement in patients with HIV infection as opposed to patients only treated with one NRTI. The data obtained from this research study highlighted the fact that the ART did not have a direct

positive effect on the LVEF, but only indirectly via its positive effect on the CD4 cell count and the viral load.

Clinical evidence of cardiac disease is usually overshadowed by manifestations in other organs, primarily the brain and lungs. As a consequence, the number of patients with AIDS, who have cardiac involvement at autopsy greatly exceeds the number with significant cardiac disease during life. Cardiac abnormalities are found at autopsy in two-thirds of patients with AIDS (Millei *et al.*, 1998). The heart is often the unrecognized target of AIDS associated lesions even in the initial phase of the AIDS outbreak (1981 – 1989) (Millei *et al.*, 1998). Although cardiac disease can occur at any stage of HIV infection, cardiac morbidity and mortality are more common in advanced stages (Millei *et al.*, 1998). The number of cardiomyopathies found at autopsies were more than those found during life. The most common reason for this finding is that the medical field is still very unaware of the fact that cardiac dysfunction occurrs in HIV/AIDS patients (Murphy *et al.,* 1998). The researcher agrees with Tavazzi (2002) who said that echocardiography is by far the best diagnostic investigation also in asymptomatic patients.

6.1. Limiting Factors

The main limiting factors that occurred during the study were:

- ⇨ Patients that passed away before follow-up procedures were done.
- ⇨ Patients that did not attend their follow-up appointments, or subsequently went to other physicians for follow-up visits.
- ⇨ The high cost of doing a viral load study precluded some patients from having this done either at the time of diagnosis or during the 3 to 6 monthly follow-up visits.
- ⇨ Some patients were admitted directly to hospital, where they usually received the blood tests, but the researcher was often unaware that they had been admitted and, as a result, did not do the LVEF study.
- ⇨ The specific time period that the patients were on a specific regimen of ART was not always known. This was due to a few reasons:
  - The fact that the patients did not always attend their follow-up appointments as scheduled.
  - Some patients stopped their ART due to side effects, financial implications and other personal reasons at any time between the follow-up visits, and usually for an unknown period.

- Patients changed from one physician to another.
- Some patients shared their monthly ART with their spouse, friends or partners. In these cases the physician could never be sure about the dosage and the time intervals at which the patient took the ART.
- Some patients became mentally confused and were not able to take the ART as prescribed. Their caretakers did not always know how the ART should be given.
- Some patients were not willing to be honest with the physician about the way he/she was taking the ART, if they were taking it at all.

6.2. Unanswered Questions that Arose

- ⇨ Why was the number of cases that were on no ART with viral load ≤100 000 RNA copies/ml and with below normal LVEF (≤52%) greater than the number of cases that were on ART (see figure 5.14)?
- ⇨ Why was the percentage of cases with below normal LVEF (≤52%) less at the CD4 cell count cut-off point of 50 cells/ml than at the CD4 cell count cut-off point of 100 cells/ml (see figure 5.4)?

6.3. Shortcomings of this Research Project and Recommendations for Possible Future Research

The study was possibly biased because a certain portion of the population was undoubtedly excluded from the study. This includes patients who do not have a medical aid or the financial capacity to consult a physician in private practice.

Recommendations for future research will be:

- ⇨ To extend a similar research project to include patients who consult public health facilities, thus excluding possible bias owing to financial implications.
- ⇨ To compile a similar research project over a longer period of time.
- ⇨ To compare the influence of other variables on the CD4 cell count, viral load and LVEF. Examples of such variables would be body mass index and other opportunistic infections.
- ⇨ To compile a similar research project, with participants who are under the supervision of a research group. This can help to monitor the patients' daily ART intake. This will result in research in which the time periods for specific ART regimens are known.

- ⇨ To compile a research project to test the possibility of a cardiotoxic effect of certain ART.
- ⇨ To evaluate the extent of increase or decrease of CD4 cell count, viral load and LVEF, when the patients were on ART or on no ART over a period of time.

Chapter 7 Conclusion

This research project showed that there is a direct proportional relationship between the CD4 cell count and the LVEF. The antiretroviral therapy had a direct positive influence on the CD4 cell count. Since the ART increased the CD4 cell count and the CD4 cell count is directly proportional to the LVEF, then the ART will indirectly have a positive influence on the LVEF.

The occurrence of the LVEF below normal occurred most frequently when the CD4 cell count was ≤100 cells/ml. Of all the patients whose LVEF was below normal, most were on no antiretroviral therapy.

The viral load was shown to have an indirect proportional relationship to the LVEF. Antiretroviral therapy had a positive effect on the viral load (except for those cases in which the patient developed resistance to the ART). Since the successful use of ART caused the viral load to decrease and the viral load and the LVEF are indirectly proportional to each other, one could say that the ART does not have a direct positive influence on the LVEF, but only via the viral load.

The occurrence of the LVEF being below normal occurred most frequently when the viral load was ≥100 000 RNA copies/ml. Of all the patients whose LVEF was below normal, most were on antiretroviral therapy, up to the viral load cut-off point of ≤100 000 RNA copies/ml. When the viral load exceeded the cut-off point of 100

000 RNA copies/ml, the patients who were on no ART with a LVEF below normal exceeded those who were on ART.

To conclude, this study confirms the findings of several previous authors (Currie *et al.,* 1998; Schlant & Alexander, 1994; Lipshultz *et al.,* 1998; Barbaro 1998b; Hivdent, 1998; Millei *et al.,* 1998; Murphy, 1999; Rerkpattanapipat *et al.,* 2000; Pugliese *et al.,* 2000; Acierno, 1989; Warkentin, 1998; Yunis *et al.*, 1998) in showing the prevalence of left ventricular dysfunction and dilated cardiomyopathy among patients with HIV infection and AIDS. The prevalence is higher in patients with advanced disease, the most significant cut-off points being a CD4 cell count at or below 100 cells/ml and a viral load above 100 000 RNA copies/ml. The study also showed the positive impact of ART on left ventricle function, where by reducing the viral burden and therefore allowing the CD4 cell count to rise, the left ventricle function improved. Most of the patients with impaired left ventricular function were asymptomatic.

The physician treating patients with HIV infection should therefore have a high index of suspicion for cardiac dysfunction. Careful clinical examination is, as always, of utmost importance. Echocardiographic examination in patients with CD4 cell counts at or below 100 will

increase the diagnostic field and therefore ensure that patients get appropriate medication and treatment.

Chapter 8 References

Acierno LJ. Journal of the American College of Cardiology (JACC). *Cardiac Complications in Acquired Immunodeficiency Syndrome (AIDS): A Review.* 1989;13(5):1144–1154.

Allen DM, Simela NP, Makubalo L. Southern African Journal of HIV Medicine. *Epidemiology of HIV/AIDS in South Africa.* 2000;Launch Issue:9–11.

Altman DG, Machin D, Bryant TH, Gardner MJ. *Statistics With Confidence.* JW Arrowsmith Ltd (eds), Bristol. 2001:48–49.

Anderson DW, Virmani R. Human Pathology. *Emerging Patterns of Heart Disease in Human Immunodeficiency Virus Infection.* 1990;21:253–259.

Anderson J, Armstead R, Maker AC, Barr D, *et al.* Annals of Internal Medicine. *Epidemiology of HIV/AIDS in South Africa.* 2000; Launch Issue: 9–11.

Anderson RH, Oldershaw PJ, Dawson JR, Rowland E. *Clinician's Illustrated Dictionary of Cardiology.* Anderson RH, Oldershaw PJ (eds). Science Press, London, UK. 3rd Edition, 1993.

Barbaro G, Di Lorenzo G, Soldini M, Giancaspro G, Grisorio B, Pellicelli A, Barbarini G. Circulation. *Intensity of Myocardial Expression of Inducible Nitric Oxide Synthesis Influences the Clinical Course of Human Immunodeficiency Virus-Associated Cardiomyopathy.* 1999,100:933–939(a).

Barbaro G, Di Lorenzo G, Grisoria B, Barbarini G. The New England Journal of Medicine. *Incidence of Dilated Cardiomyopathy and Detection of HIV in Myocardial Cells of HIV-Positive Patients.* 1998,339(16):1093–1099(b).

Coudray N, de Zuttere D, Force D, *et al.* European Heart Journal. *Left Ventricular Diastolic Dysfunction in Asymptomatic and Symptomatic Human Immunodeficiency Virus Cariers: an Echocardiographic Study.* 1995;16:61–67.

Currie PF, Jacob AJ, Foreman AR, Elton RA, Brettle RP, Boon NA. Heart. *Cardiac Autoimmunity in HIV Related Heartmuscle Disease.* 1998;79(6):599–604.

De Castro S, Migliau G, Silvestri A, D'Amati G, Giannantoni P, Cartoni D, Kol A, Vullo V, Cirelli A. European Heart Journal. *Heart Involvement in AIDS: A Prospective Study During Various Stages of the Diesease.* 1992;13:1452–1459.

Echo by Web (Last updated 2003-01-02). *Atlases and Reference Material for Echocardiography* [online], State University of Milan. Available from: <http://www.echobyweb.com> [Accessed 12 September 2003].

Fauci AS, Bartlett JG, Goosby EP. Annals of Internal Medicine. *Guidelines for the Use of Antiretroviral Agents in HIV-Infected Adults and Adolescents.* 1998,128(12.2):1079.

Hakim JG, Matenga JA, Siziya S. Heart. *Myocardial Dysfunction in Human Immunodeficiency Virus Infection: An Echocardiographic Study of 157 Patients in a Hospital in Zimbabwe.* 1996;76:161–165.

HIVdent. Medical considerations. *Clinical Management of the HIV-Infected Adult Disease-specific Protocols: HIV-Related Cardiomyopathy* [online]. 1998. Available from: <www.hivdent.org/medmid/middsp012.htm> [Accessed 21 December 2001].

Ince CS. Medscape Cardiology. *Evidence-Based Cardiology Incidence of Dilated Cardiomyopathy and Detection of HIV in Myocardial Cells of HIV-Positive Patients* [online] Johns Hopkins Hospital, Baltimore. 1999. Available from: <http://www.medscape.com/Medscape/cardiology/EBC/1999/02-.../pnt-mc0217.01.ince.htm> [Accessed 21 December 2001].

Kenny D, Shen LP, Kolberg JA. Journal of Histochemistry and Cytochemistry. *Detection of Viral Infection and Gene Expression in Clinical Tissue Specimens Using Branched DNA (bDNA) In Situ Hybridization.* 2002;50:1219–1227.

Lipshultz SE. New England Journal of Medicine. *Editorial: Dilated Cardiomyopahty in HIV-Infected Patients.* 1998;339(16):1153–1154.

Longo Mbenza B, Tonduangu K, Muvova D, Phuati MB, Seghers KV, Kestelot H. Arch Mal Coeur Vaiss. *A Clinical Study of Cardiac Manifestations Related to AIDS in Kinshasa.* 1995;88:1437–1443.

Makotoko M. Specialist Physician and Cardiologist. Personal conversation with the author in November 2003. Bloemfontein, South Africa. November 2003.

Millei J, Grana D, Alonso GF, Matturi L *et al.* Clinical Cardiology. *Cardiac Involvement in Acquired Immunodeficiency Syndrome – A Review to Push Action.* 1998;21:465–472.

Miller S (innovirInstitute). *HIV Treatment Protocol.* Bankmed Medical Aid Scheme. South Africa. 1st Edition, 2000(a).

Miller S (innovirInstitute). *HIV/AIDS Information Manual.* Bankmed Medical Aid Scheme. South Africa. 1st Edition, 2000(b).

Miller S. South African HIV Clinicians Society. *Clinical Guidelines to Antiretroviral Therapy in Adults.* 2002; 25th June.

Montaner JSG, Reiss P, Cooper D, Vella S, Harris M, *et al.* The Journal of the American Medical Association (JAMA). *A Randomized, Double-blind Trial Comparing Combinations of Nevirapine, Didanosine, and Zidovudine for HIV-Infected Patients: The INCAS Trial.* 1998;279(12):930–937.

Murphy S. University of Cape Town: Clinical Cardiology. *HIV and the Heart.* [Lecture on 13 June 1999].

Principles of ICH GCP [online]. Available from: <http://www.ncehr-cnerh.org/english/gcp/principles.html> [Accessed 22 January 2002].

Pugliese A, Isnardi D, Saini A, Scarabelli T, Raddino R, Torre D. Journal of Infection. *Impact of Highly Active Antiretroviral Therapy in HIV-positive Patients with Cardiac Involvement.* 2000;40:282–284.

Rerkpattanapipat P, Wongpraparut N, Jacobs LE, Kotler MN. Archives of Internal Medicine. *Cardiac Manifestations of Acquired Immunodeficiency Syndrome.* 2000;160:602–608.

Schlant RC, Alexander RW. *Hurst's The Heart.* Schlant RC, Alexander RW (eds). McGraw-Hill INC Health Professions Division, New York (USA). 8th Edition; 1994; 2: 1953 – 1960; 1609–1619.

Southern African HIV Clinicians Society. Southern African Journal of HIV Medicine. *Clinical Guidelines: Antiretroviral Therapy in Adults, June 2002 Version.* 2002;July:22–29.

Smart T. GMHC Treatment Issues. *Viral Load Comes of Age.* 1996;10:6–7.

Tavazzi L. The European Cardiologist – Journal by Fax. *Cardiomyopathy in Acquired Immunodeficiency Syndrome.* 2002;7(66):1.

UNAIDS/WHO Epidemiological Fact Sheet. *Estimated Number of People Living with HIV/AIDS.* 2002 Update p2 – South Africa. Available from: <http://www.who.int/emc-hiv/fact_sheets> [Accessed December 2002].

University of the Free State: Department of Hemaetology. *The normal values for the T, B and NK Lymphocytes as compiled by the Department of Hemaetology.* 1997/8

Van Dyk A. *Hiv/Aids Care and Counselling: A multidisciplinary approach.* Roger Loveday (ed). Pearson Education, South Africa. 2nd Edition, 2001.

Warkentin DL. Healthinformatics. *AIDS and Heart Disease* [online]. 1998. Available from: <http://www.healthinformatics.com/docs/english/CA/aidheart.car.asp> [Accessed 21 December 2001].

Weyman AE. *Principles and Practice of Echocardiography.* Bussy RK, Lazar T, Klass F. Lea, Febiger (eds). Pennsylvania (USA). 2nd Edition, 1994; 781 – 823.

World Health Organisation (WHO). WHO Initiative on HIV/AIDS and Sexually Transmitted Infections (STI). *Principles of Antiretroviral Therapy.* 2002, 25th June.

World Health Organisation (WHO). WHO Regional Office for South-East Asia. *Fact Sheets on Antiretroviral Drugs.* 2003, 8th May.

World Medical Association. *Declaration of Helsinki* [online]. 2002. Available from: <http://www.faseb.org/aevo/helsinki.htm> [Accessed 6 October 2002].

Yunis NA, Stone VE. Journal of Acquired Immune Deficiency Syndrome and Human Retrovirology. *Cardiac Manifestations of HIV/AIDS: A Review of Disease Spectrum and Clinical Management.* 1998;18(2):145–154.

Appendices

Appendix A: Results

Table A.1: Percentage of cases with LVEF ≤ 52% when the CD4 cell count was at certain cut-off points.

Out of the population there were 114 cases of patients with CD4 cell counts and LVEF studies in which the LVEF study was below normal (≤52%), and 337 cases of patients with CD4 cell counts and LVEF studies in which the LVEF study was within the normal range (>52% - 75%). This resulted in a total of 451 cases.

The difference in the percentage was calculated via the difference between the percentage of cases with below normal LVEF in cases with a CD4 cell count less or equal to (≤) the cut-off point, and a CD4 cell count greater than the cut-off point.

The cut-off points for the CD4 cell counts were 50, 100, 150, 200, 250, 300, 350, >350 cells/ml. The >350 cells/ml cut-off point (i.e. 0 - >350) included all the cases defined above.

Part 1:

CD 4 cell count (Cells/ml)	LVEF ≤52%	LVEF >52%	Total
Total	114	337	451

LVEF: Left ventricle ejection fraction (normal value: >52% - 75%)

Calculations:

- ⇨ Percentage of cases with below normal LVEF (≤52%) in all the above defined cases: 114 ÷ (114 + 337) x 100= 25.28%.

Part 2:

CD 4 cell count (Cells/ml)	LVEF ≤52%	LVEF >52%	Total
≤50	33	59	92
>50	81	278	359
Total	114	337	451

LVEF: Left ventricle ejection fraction (normal value: >52% - 75%)

Calculations:

- ⇨ Percentage of cases with below normal LVEF (≤52%) in cases with CD4 cell count ≤50 is: 33 ÷ (33 + 59) x 100= 35.87%.
- ⇨ Percentage of cases with below normal LVEF (≤52%) in cases with CD4 cell count >50 is: 81 ÷ (81 + 278) x 100= 22.56%.
- ⇨ Difference: 13.3%.
- ⇨ 95% Wilson confidence interval for the % difference: [3.2% ; 22.6%]

Part 3:

CD 4 cell count (Cells/ml)	LVEF ≤52%	LVEF >52%	Total
≤100	57	86	143
>100	57	251	308
Total	114	337	451

LVEF: Left ventricle ejection fraction (normal value: >52% - 75%)

Calculations:

- ⇨ Percentage of cases with below normal LVEF (≤52%) in cases with CD4 cell count ≤100 is: 57 ÷ (57 + 86) x 100= 39.86%.
- ⇨ Percentage of cases with below normal LVEF (≤52%) in cases with CD4 cell count >100 is: 57 ÷ (57 + 251) x 100= 18.51%.
- ⇨ Difference: 21.4%
- ⇨ 95% Wilson confidence interval for the % difference: [12.4% ; 30.4%]

Part 4:

CD 4 cell count (Cells/ml)	LVEF ≤52%	LVEF >52%	*Total*
≤150	70	119	189
>150	44	218	262
Total	114	337	451

LVEF: Left ventricle ejection fraction (normal value: >52% - 75%)

Calculations:

- ⇨ Percentage of cases with below normal LVEF (≤52%) in cases with CD4 cell count ≤150 is: 70 ÷ (70 + 119) x 100 = 37.04%.
- ⇨ Percentage of cases with below normal LVEF (≤52%) in cases with CD4 cell count >150 is: 44 ÷ (44 + 218) x100 = 16.79%.
- ⇨ Difference: 20.2%
- ⇨ 95% Wilson confidence interval for the % difference: [12.0% ; 28.4%]

Part 5:

CD 4 cell count (Cells/ml)	LVEF ≤52%	LVEF >52%	*Total*
≤200	77	146	223
>200	37	191	228
Total	114	337	451

LVEF: Left ventricle ejection fraction (normal value: >52% - 75%)

Calculations:

- ⇨ Percentage of cases with below normal LVEF (≤52%) in cases with CD4 cell count ≤200 is: 77 ÷ (77 + 146) x 100 = 34.53%.
- ⇨ Percentage of cases with below normal LVEF (≤52%) in cases with CD4 cell count >200 is: 37 ÷ (37 + 191) x 100 = 16.23%.
- ⇨ Difference: 18.3%
- ⇨ 95% Wilson confidence interval for the % difference: [10.3% ; 26.0%]

Part 6:

CD 4 cell count (Cells/ml)	LVEF ≤52%	LVEF >52%	*Total*
≤250	82	171	253
>250	32	166	198
Total	114	337	451

LVEF: Left ventricle ejection fraction (normal value: >52% - 75%)

Calculations:

- ⇨ Percentage of cases with below normal LVEF (≤52%) in cases with CD4 cell count ≤250 is: 82 ÷ (82 + 171) = 32.41%.
- ⇨ Percentage of cases with below normal LVEF (≤52%) in cases with CD4 cell count >250 is: 32 ÷ (32 + 166) = 16.16%.
- ⇨ Difference: 16.2%
- ⇨ 95% Wilson confidence interval for the % difference:[8.3% ; 23.7%]

Part 7:

CD 4 cell count (Cells/ml)	LVEF ≤52%	LVEF >52%	*Total*
≤300	93	201	294
>300	21	136	157
Total	114	337	451

LVEF: Left ventricle ejection fraction (normal value: >52% - 75%)

Calculations:

- ⇨ Percentage of cases with below normal LVEF (≤52%) in cases with CD4 cell count ≤300 is: 93 ÷ (93 + 201) = 31.63%.
- ⇨ Percentage of cases with below normal LVEF (≤52%) in cases with CD4 cell count >300 is: 21 ÷ (21 + 136) = 13.38%.
- ⇨ Difference: 18.35%
- ⇨ 95% Wilson confidence interval for the % difference: [10.3% ; 25.4%]

Part 8:

CD 4 cell count (Cells/ml)	LVEF ≤52%	LVEF >52%	*Total*
≤350	96	231	327
>350	18	106	124
Total	114	337	451

LVEF: Left ventricle ejection fraction (normal value: >52% - 75%)

Calculations:

- ⇨ Percentage of cases with below normal LVEF (≤52%) in cases with CD4 cell count ≤350 is: 96 ÷ (96 + 231) = 29.36%.
- ⇨ Percentage of cases with below normal LVEF (≤52%) in cases with CD4 cell count >350 is: 18 ÷ (18 + 106) = 14.52%.
- ⇨ Difference: 14.8%.
- ⇨ 95% Wilson confidence interval for the % difference: [6.2% ; 22.1%].

Table A.2: Percentage of cases with LVEF ≤ 52% when the viral load was at certain cut-off points.

Out of the population there were 79 cases of patients with viral load studies and LVEF studies in which the LVEF study was below normal (≤52%), and 286 cases of patients with viral load studies and LVEF studies in which the LVEF study was within the normal range (>52% - 75%). This resulted in a total of 365 cases.

The difference in the percentage of cases was calculated as the difference between the percentage of cases with below normal LVEF in cases with viral load larger than the cut-off point and the viral load less or equal to (≤) the cut-off point.

The ranges in which the viral load (PCR-method) can be measured, are between <400 RNA copies/ml (or in some cases <50 RNA/copies/ml when the doctor requested the ultra HIV PCR viral load test) and >750 000 RNA copies/ml. The cut-off points in this study were ≤1 000 (i.e. $\leq 10^3$), 10 000 (i.e. 10^4), 100 000 (i.e. 10^5) and 1 000 000 (i.e. 10^6). Therefore the lowest value of viral load (<400 RNA copies/ml or <50 RNA copies/ml in some cases) was included in the ≤1 000 cut-off point, and the highest value of viral load (>750 000 RNA copies/ml) was included in the cut-off point of

<1 000 000. The cut-off point of <1 000 000 will therefore include all the above defined cases (i.e. 0 – 1 000 000).

Part 1

Viral load (RNA copies/ml)	LVEF ≤52%	LVEF >52%	*Total*
Total	79	286	365

LVEF: Left ventricle ejection fraction (normal value: >52% - 75%)

Calculations:

- ⇨ Prevalence of below normal LVEF (≤52%) in cases with viral load ≤1 000 000 is: 79 ÷ (79 + 286) x 100 = 21.64%.

Part 2:

Viral load (RNA copies/ml)	LVEF ≤52%	LVEF >52%	*Total*
≤ 1 000	15	62	77
> 1 000	64	224	288
Total	79	286	365

LVEF: Left ventricle ejection fraction (normal value: >52% - 75%)

Calculations:

- ⇨ Percentage of cases with below normal LVEF (≤52%) in cases with viral load ≤1 000 is: 15 ÷ (15 + 62) x 100 = 19.58%.
- ⇨ Percentage of cases with below normal LVEF (≤52%) in cases with viral load >1 000 is: 64 ÷ (64 + 224) x 100 = 22.22%.
- ⇨ Difference: 2.37%
- ⇨ 95% Wilson confidence interval for the % difference: [-8.4% ; 11.7%]

Part 3:

Viral load (RNA copies/ml)	LVEF ≤52%	LVEF >52%	*Total*
≤ 10 000	19	110	129
> 10 000	60	176	236
Total	79	286	365

LVEF: Left ventricle ejection fraction (normal value: >52% - 75%)

Calculations:

- ⇨ Percentage of cases with below normal LVEF (≤52%) in cases with viral load ≤10 000 is: 19 ÷ (19 + 110) x 100 = 14.73%.

- ⇨ Percentage of cases with below normal LVEF (≤52%) in cases with viral load >10 000 is: 60 ÷ (60 + 176) x 100 = 25.42%.
- ⇨ Difference: 10.69%
- ⇨ 95% Wilson confidence interval for the % difference: [1.9% ; 18.5%]

Part 4:

Viral load (RNA copies/ml)	LVEF ≤52%	LVEF >52%	*Total*
≤100 000	27	172	199
>100 000	52	114	166
Total	79	286	365

LVEF: Left ventricle ejection fraction (normal value: >52% - 75%)

Calculations:

- ⇨ Percentage of cases with below normal LVEF (≤52%) in cases with viral load ≤100 000 is: 27 ÷ (27 + 172) x 100 = 13.57%.
- ⇨ Percentage of cases with below normal LVEF (≤52%) in cases with viral load >100 000 is: 52 ÷ (52 + 114) x 100 = 31.33%.
- ⇨ Difference: 17.76%
- ⇨ 95% Wilson confidence interval for the % difference: [9.2% ; 26.2%].

Table A.3: Percentage of cases that were on ART versus those on no ART at certain CD4 cell count cut-off points and with LVEF below normal (≤ 52%).

Out of the population there were 114 cases of patients with CD4 cell counts and LVEF studies in which the LVEF study was below normal (≤52). Out of these cases 67 cases were on no ART and 47 cases were on any combination of ART.

The difference in the percentage was calculated as the difference between the percentage of cases with below normal LVEF in cases with a CD4 cell count less or equal to (≤) the cut-off point of those cases that were on no ART and those cases that were on any combination of ART.

The cut-off points for the CD4 cell count were 50, 100, 150, 200, 250, 300, 350, >350 cells/ml. The >350 cells/ml cut-off point (i.e. 0 - >350) included all the cases defined above.

Part 1:

	CD4 cell count (cells/ml)
	Total
No ART	67 (58.8%)
Any combination of ART	47 (41.2%)
Total	114

Part 2:

	CD4 cell count (cells/ml)		
	≤50	**>50**	***Total***
No ART	20	47	67
Any combination of ART	13	34	47
Total	33	81	114

Calculations:

- ⇨ Percentage of cases with CD4 cell count ≤50 cell/ml in cases that were on no ART is: 20 ÷ (20 + 47) x 100 = 29.85%
- ⇨ Percentage of cases with CD4 cell count ≤50 cell/ml in cases that were on any combination of ART is: 13 ÷ (13 + 34) x 100= 27.66%
- ⇨ Difference: 2.2%
- ⇨ 95% Wilson confidence interval for the % difference: [-14.9% ; 18.1%]

Part 3:

	CD4 cell count (cells/ml)		
	≤100	**>100**	***Total***
No ART	38	29	67
Any combination of ART	19	28	47
Total	57	57	114

Calculations:

- ⇨ Percentage of cases with CD4 cell count ≤100 cell/ml in cases that were on no ART is: 38 ÷ (38 + 29) x 100 = 56.72%
- ⇨ Percentage of cases with CD4 cell count ≤100 cell/ml in cases that were on any combination of ART is: 19 ÷ (19 + 28) x 100 = 40.43%
- ⇨ Difference: 16.3%
- ⇨ 95% Wilson confidence interval for the % difference: [-2.3% ; 33.3%.]

Part 4:

	CD4 cell count (cells/ml)		
	≤150	**>150**	***Total***
No ART	44	23	67
Any combination of ART	26	21	47
Total	70	44	114

Calculations:

- ⇨ Percentage of cases with CD4 cell count ≤150 cell/ml in cases that were on no ART is: 44 ÷ (44 + 23) x 100 = 65.67%
- ⇨ Percentage of cases with CD4 cell count ≤150 cell/ml in cases that were on any combination of ART is: 26 ÷ (26 + 21) x100 = 55.32%
- ⇨ Difference: 10.4%
- ⇨ 95% Wilson confidence interval for the difference: [-7.5% ; 27.8%]

Part 5:

	CD4 cell count (cells/ml)		
	≤200	**>200**	***Total***
No ART	48	19	67
Any combination of ART	29	18	47
Total	77	37	114

Calculations:

- ⇨ Percentage of cases with CD4 cell count ≤200 cell/ml in cases that were on no ART is: 48 ÷ (48 + 19) x 100 = 71.64%
- ⇨ Percentage of cases with CD4 cell count ≤200 cell/ml in cases that were on any combination of ART is: 29 ÷ (29 + 18) x 100 = 61.7%
- ⇨ Difference: 9.9%
- ⇨ 95% Wilson confidence interval for the difference: [-7.2% ; 27%]

Part 6:

	CD4 cell count (cells/ml)		
	≤250	**>250**	***Total***
No ART	53	14	67
Any combination of ART	29	18	47
Total	82	32	114

Calculations:

- ⇨ Percentage of cases with CD4 cell count ≤250 cell/ml in cases that were on no ART is: 53 ÷ (53 + 14) x 100 = 79.1%
- ⇨ Percentage of cases with CD4 cell count ≤250 cell/ml in cases that were on any combination of ART is: 29 ÷ (29 + 18) x 100 = 61.7%
- ⇨ Difference: 17.4%
- ⇨ 95% Wilson confidence interval for the % difference: [0.6% ; 33.8%]

Part 7:

	CD4 cell count (cells/ml)		
	≤300	**>300**	***Total***
No ART	58	9	67
Any combination of ART	35	12	47
Total	93	21	114

Calculations:

- ⇨ Percentage of cases with CD4 cell count ≤300 cell/ml in cases that were on no ART is: 58 ÷ (58 + 9) x 100 = 86.57%
- ⇨ Percentage of cases with CD4 cell count ≤300 cell/ml in cases that were on any combination of ART is: 35 ÷ (35 + 12) x 100 = 74.47%
- ⇨ Difference: 12.1%
- ⇨ 95% Wilson confidence interval for the difference: [-2.4% ; 27.4%]

Part 8:

	CD4 cell count (cells/ml)		
	≤350	**>350**	***Total***
No ART	60	7	67
Any combination of ART	36	11	47
Total	96	18	114

Calculations:

- ⇨ Percentage of cases with CD4 cell count ≤350 cell/ml in cases that were on no ART is: 60 ÷ (60 + 7) x 100 = 89.55%
- ⇨ Percentage of cases with CD4 cell count ≤350 cell/ml in cases that were on any combination of ART is: 36 ÷ (36 + 11) x 100 = 76.6%
- ⇨ Difference: 13.0%
- ⇨ 95% Wilson confidence interval for the % difference: [-0.8% ; 27.8%]

Table A.4: Percentage of cases that were on ART versus those on no ART at certain viral load cut-off points and below normal LVEF (≤52%).

Out of the population there were 79 cases of patients with viral load studies and LVEF studies in which the LVEF study was below normal (≤52%). From the 79 cases, 47 cases were on no ART and 32 cases were on any combination of ART.

The difference in the percentage was calculated as the difference between the percentage of cases with below normal LVEF in cases with viral load smaller or equal to the cut-off point for the cases that were on any combination of ART and for the cases that were on no ART.

The ranges in which the viral load (PCR-method) can be measured, are <400 RNA copies/ml (in some cases <50 RNA copies/ml when the doctor requested the ultra HIV PCR viral load test) up to >750 000 RNA copies/ml. The cut-off points in this study were ≤1 000 (i.e. $\leq 10^3$), 10 000 (i.e. 10^4), 100 000 (i.e. 10^5) and 1 000 000 (i.e. 10^6). Therefore the lowest value of viral load (<400 RNA copies/ml) was included in the <1 000 cut-off point, and the highest value of viral load (>750 000 RNA copies/ml) was included in the cut-off point of <1 000 000. The cut-off point of <1 000 000 would therefore include all the above defined cases (i.e. 0 – 1 000 000).

Part 1:

	Viral Load (RNA copies/ml) ***Total***
No ART	47
Any combination of ART	32
Total	79

Calculations:

- ⇨ Of the 79 cases with below normal LVEF, 47 (59.5%) used no ART and 32 (40.5%) used any combination of ART.

Part 2:

	Viral Load (RNA copies/ml)		
	≤1 000	**>1 000**	***Total***
No ART	1	46	47
Any combination of ART	14	18	32
Total	15	64	79

Calculations:

- ⇨ Percentage of cases with viral load ≤1 000 RNA copies/ml in cases that were on no ART is: 1 ÷ (1 + 46) x 100 = 2.13%
- ⇨ Percentage of cases with viral load ≤1 000 RNA copies/ml in cases that were on any combination of ART is: 14 ÷ (14 + 18) x 100 = 43.75%
- ⇨ Difference: 41.6%
- ⇨ 95% Wilson confidence interval for the % difference: [23.6% ; 58.6%]

Part 3:

	Viral Load (RNA copies/ml)		
	≤10 000	**>10 000**	***Total***
No ART	3	44	47
Any combination of ART	16	16	32
Total	19	60	79

Calculations:

- ⇨ Percentage of cases with viral load ≤10 000 RNA copies/ml in cases that were on no ART is: 3 ÷ (3 + 44) x 100 = 6.38%

- ⇨ Percentage of cases with viral load ≤10 000 RNA copies/ml in cases that were on any combination of ART is: 16 ÷ (16 + 16) x 100= 50%
- ⇨ Difference: 43.62%
- ⇨ 95% Wilson confidence interval for the % difference: [9.5% ; 60.5%]

Part 4:

	Viral Load (RNA copies/ml)		
	≤100 000	**>100 000**	***Total***
No ART	10	37	47
Any combination of ART	17	15	32
Total	27	52	79

Calculations:

- ⇨ Percentage of cases with viral load ≤100 000 RNA copies/ml in cases that were on no ART is: 10 ÷ (10 + 37) x 100 = 21.28%
- ⇨ Prevalence of viral load ≤100 000 RNA copies/ml in cases that were on any combination of ART is: 17 ÷ (17 + 15) x 100 = 53.13%.
- ⇨ Difference: 31.85%.
- ⇨ 95% Wilson confidence interval for the % difference: [10.3% ; 50.4%].

Appendix B: Consent Forms and Practice Registration Form.

B.1. Subject Information Sheets and Statements of Informed Consent.

B.1.1. Informed Consent

I, _____, date of birth_____,
Patient's name in block letters

hereby consent to participate in the research trial:

The influences of CD4 count, Viral Load and Antiretroviral Treatment on the Left Ventricle Ejection Fraction of adult HIV/AIDS patients.

Dr. M Makotoko has informed me in detail and in a comprehensible manner about the nature, importance and scope of the trial. I have read and understood the text of the patient information and this declaration of consent. My questions were answered by dr. M. Makotoko in sufficient detail. I understand that I reserve the right to withdraw my consent at any time without suffering any disadvantages and without my decision influencing my doctor-patient relationship.

With my signature I also consent that mrs S Steenkamp may inspect my medical records for the purpose of the trial.
My identity will be treated with absolute confidentiality and will not be imparted to third parties.

_____
Place

_____ _____
Date Patient's signature

_____
Place

_____ _____
Date Doctor's signature

B.1.2. Patient Information

Thank you for agreeing to take part in this trial.

I should like to point out that your participation is voluntary. You have the right to withdraw your consent to participate at any time without giving reasons and without any disadvantages arising in terms of further medical care.

Your data will be passed on for scientific evaluation, but this will be entirely anonymous.

For this trial to take place, we will need you to consent to the following:

1. That your blood results and disease are made known to mrs S Steenkamp (clinical technologist) for the sole purpose of the trial.
2. That your treatment plan is made known to mrs S Steenkamp (clinical technologist) for the sole purpose of her research project.
3. That mrs S Steenkamp may perform an echocardiogram on you to evaluate your heart status. This will be done free of charge *if* the echocardiogram is for the sole purpose of the research project.
4. That the above-mentioned data will be used for the research project.

Your medical records will be treated with absolute confidentiality. Your name will not be made public. The data will be linked to a number and NOT to your personal detail. Thus nobody will know to whom the data belongs.

We thank you very much for your co-operation. I will be glad to answer any other questions you might have.

Dr. M. Makotoko

B.1.3. Tsebiso ya tumellano

Nna _____, letsatsi la tswalo_____. Ke fana ka tumello yaka ho nka karolo dipatlisisong tsena:

Dr. M. Makotoko o ile a ntlhalosetsa ka botlalo bohlokwa le tsela eo dipatlisiso di tlilo etswang ka yona. Ke utluisisa hore patlisiso e na e batla eng, le hore tumello ya ka ya ho nka karolo e bolelang. Dipotso tsaka di ile tsa arajwa ka botlalo ke Dr. Makotoko. Ke utluisisa hore ke na le tokelo ya ho tlohela hare ho sebaka ka ntle le ho fana ka tlhaloso, mme ho tlohela haka ho ke ke ha etsa hore tshebeletso yaka e be tsuong.

Ho saena haka ho fana ka tumello ya hore Mme S. Steenkamp aka bala mangolo aka a ngaka se bakeng sa dipatlisiso. Mabitso le manyolo aka a tla sebeletswa ka sephiri a sa fuwe batho babang.

_____

Tulo

_____

Letsatsi

_____

Tshaene ya monka karolo

_____

_____

Tulo

_____

Letsatsi

_____

Tshaeno ya Ngaka

B.1.4. Tlhalosetso ho Mokudi

Ke leboha ha o dumetse ho nka karolo patlisisong ena.
Ke lakatsa ho supa hore ho nka karolo ha hao ke boithaopi. O na le tokelo ya ho fetola maikutlo ho nkeng karolo nako le nako, kantle le ho fana ka mabaka. Tshebeletso yeo re o fang yona e ke ke ya angoa ke ho tlohela ha hao.

Lipalo-palo tsa hao di tla fetisetswa ditekong tsa tlhaho (Scientific) empa sena setla etswa ho sa sebediswe mabitso a hao.

Sebakeng sa patlisiso ena ho nka sebaka, retla lakatsa hore o tsepamise maikutlo a hao ntlheng tsena:

1. Sephetho sa madi a hao le lefu la hao ditla tsejwa ke mofumahadi S Steenkamp setsebi sa botegonologi bakeng sa patlisiso ena.
2. Boemo ba bokudi ba hao bo tla tsejwa ke mofumahadi S. Steenkamp, setsibi sa botegonologi sebakeng sa ho ntshetsa pele dipathisiso tsena.
3. Mofumahadi S. Steenkamp o tla etsa tlhathlobo ea pelo ea hao ea echocardiogram ntle le tefello.
4. Dintlha tse ka hodimo ditla sebediswa bakeng sa diteko tsa porojeke.

Ngodiso ya phodiso etla etswa ka lekunutu. Lebitso la hao le dintlha tsa hao ditla bapiswa le nomoro ya hao empa ese ya mosebetsi. Ha ho mang kapa mang yatla tseba hore dipalo-palo tseo ketsa mang.

Re leboha haholo bakeng la tshebedisano-mmoho ya hao le rona. Ke tla thabela ho araba potso efe kappa efe e tswang ho wena.

Dr M MAKOTOKO

B.1.5. Toestemmingsvorm

Ek, _____, geboortedatum _____ gee hiermee my toestemming om deel te neem aan die navorsingsprojek:

"The influence of CD4 cell count, Viral load and Antiretroviral Therapy on the Left Ventricle Ejection Fraction of Adult HIV/AIDS patients."

Dr Makotoko het my volledig ingelig en in verstaanbare terme die wyse, die belangrikheid en die omvang van die studie verduidelik. Ek het die inligtingstuk gelees en dit verstaan en ook die verklaring van toestemming. My vrae sal so deeglik as moontlik beantwoord word deur Dr Makotoko. Ek verstaan dat ek ter eniger tyd kan onttrek van die studie sonder enige benadeling van my dokter-pasiënt verhouding.

Met my handtekening gee ek ook toestemming dat Mev S Steenkamp my mediese rekords mag sien en gebruik vir die studie. My identiteit en pasiëntinligting sal konfidensieël hanteer word en nie aan 'n derde party deurgegee word nie.

_____
Plek

_____
Datum

_____
Pasiënt se handtekening

_____

_____
Plek

_____
Datum

_____
Dokter se handtekening

B.1.6. Pasiënt Inligtingstuk

Dankie dat u bereid is om deel van die projek te wees.

Ek wys aan u uit dat u deelname vrywillig is. U het die reg om enige tyd van die projek te onttrek sonder om 'n rede te verskaf. U sal ook nie benadeel word in enige verdere behandeling nie.

U data en inligting sal gebruik word vir wetenskaplike evaluering, waar die inligting anoniem hanteer word.

Om die studie moontlik te maak het ons u toestemming nodig vir die volgende:

1. Dat u bloeduitslae en siektetoestand bekend gemaak mag word aan Mev S Steenkamp (Kliniese Tegnoloog) vir die duur van die studie.
2. Dat u behandeling bekend gemaak mag word aan Mev S Steenkamp (Kliniese Tegnoloog) vir die duur van die studie.
3. Dat Mev S Steenkamp 'n eggokardiogram mag doen om u hartstatus te bepaal. Daar is geen koste aan die toets verbonde nie en dit word uitsluitlik vir die studie gebruik.
4. Dat al die bogenoemde inligting gebruik mag word vir die navorsingsprojek.

U mediese rekords sal konfidensieël hanteer word. U naam sal nie bekend gemaak word nie. Die data sal verbind word met 'n nommer en nie met 'n persoon nie.

Baie dankie vir u samewerking . Ek sal graag enige verdere vrae beantwoord wat u mag hê oor die studie.

Dr. M. Makotoko

B.2. Practice Registration Form

PATIENT DATA

Surname: (Mr/Mrs/Miss)_____
Full name: _____
ID Nr.: _____
REFERRED BY: _____

PERSON RESPONSIBLE FOR ACCOUNT

Surname (Mr/Mrs/Miss): _____
Full name: _____
Postal address: _____
_____ Code _____
Home address: _____
_____ Code:_____
Tel: (H) _____ (W) _____
Cell Phone: _____
Employer: _____
Medical aid name: _____Nr. _____

Date: _____**Signature** _____

Appendix C: Example of the Raw Data

Patient number	DOB	Sex	Date	Viral Load	CD4	LVEF %	NRTI	NNRTI	PI	Other
1	590828	F	2001/03/15	607 521	91		No ART			
	590828	F	2001/09/17			57	Didanosine, Stavudine	Efavirenz		
	590828	F	2001/09/18	593 824	55		Didanosine, Stavudine	Efavirenz		
	590828	F	2002/05/02	1 410 164	71		Didanosine, Stavudine	Efavirenz		
	590828	F	2002/05/03		119		Didanosine, Stavudine	Efavirenz		
	590828	F	2002/07/09		60		No ART			
	590828	F	2002/09/28		26			Efavirenz	Ritonavir, Indinavir	
	590828	F	2002/10/01			30	Didanosine, Stavudine	Efavirenz		
	590828	F	2002/10/14			47	Didanosine, Stavudine	Efavirenz		
	590828	F	2002/10/23				Didanosine, Stavudine	Efavirenz		
2	670730	M	1999/04/10		346		No ART			
	670730	M	1999/04/13	2 580			No ART			
	670730	M	1999/08/20		296		Didanosine, Stavudine			Hydroxyurea
	670730	M	1999/08/28	627			Didanosine, Stavudine			Hydroxyurea
	670730	M	1999/12/09	101 000 250		65	Didanosine, Stavudine			Hydroxyurea
	670730	M	2001/12/21	68 588 202				Efavirenz	Ritonavir, Indinavir	
	670730	M	2002/02/04			58		Efavirenz	Ritonavir, Indinavir	
	670730	M	2002/03/11	50 500 462				Efavirenz	Ritonavir, Indinavir	
	670730	M	2002/05/24		526			Efavirenz	Ritonavir, Indinavir	
	670730	M	2002/07/29	123 000				Efavirenz	Ritonavir, Indinavir	
	670730	M	2002/08/07	< 400				Efavirenz	Ritonavir, Indinavir	
	670730	M	2002/10/05	26 100 168			Lamivudine, Zidovudine	Efavirenz		
	670730	M	2003/01/05	203 000 149			Lamivudine, Zidovudine	Efavirenz		
	670730	M	2003/01/06			58	Lamivudine, Zidovudine	Efavirenz		
	670730	M	2003/01/23		79			Efavirenz	Ritonavir, Indinavir	
	670730	M	2003/02/13	399 000 123				Efavirenz	Ritonavir, Indinavir	
	670730	M	2003/02/16					Efavirenz	Ritonavir, Indinavir	
3	740516	F	2001/05/28		44		No ART			
	740516	F	2001/05/29			57	No ART			
	740516	F	2001/08/03		31		No ART			
4	571105	F	2003/04/14	17 100 314		58	No ART			
5	720203	M	2003/01/24	386 000 177			No ART			
	720203	M	2003/01/27		214		No ART			
	720203	M	2003/03/03			65	Didanosine, Stavudine	Efavirenz		

Patient number	DOB	Sex	Date	Viral Load	CD4	LVEF %	NRTI	NNRTI	PI	Other
6	640406	M	2002/05/27		63		No ART			
	640406	M	2002/07/02	> 750 000	65		Didanosine, Stavudine	Efavirenz		
	640406	M	2002/08/15			60	Didanosine, Stavudine	Efavirenz		
7	710502	F	2000/08/26		238		No ART			
	710502	F	2000/09/13	601 624	52		No ART			
	710502	F	2000/09/15			43	Lamivudine, Zidovudine			
	710502	F	2000/11/21			32	Lamivudine, Zidovudine			
	710502	F	2000/12/07	148 000			Lamivudine, Zidovudine			
	710502	F	2000/12/21	5 578 124			Lamivudine, Zidovudine	Nevirapine		
	710502	F	2001/01/16	428 000	70		Lamivudine, Zidovudine	Nevirapine		
	710502	F	2001/02/13		67		Lamivudine, Zidovudine	Nevirapine		
	710502	F	2001/02/14	295 000	6		Didanosine, Stavudine			
	710502	F	2001/07/09	675 000	119		Didanosine, Stavudine			
	710502	F	2001/08/16		187		Didanosine, Stavudine			
	710502	F	2001/09/27	164 000	126		Lamivudine, Zidovudine			
	710502	F	2002/01/21			30	Lamivudine, Zidovudine			
	710502	F	2002/09/07				Lamivudine, Zidovudine			
8	731002	F	2002/08/28		183	67	No ART			
	731002	F	2002/09/03	26 100			No ART			
9	790323	F	2001/08/17	364 887	15		No ART			
	790323	F	2002/08/20		331		Didanosine, Stavudine			
	790323	F	2002/12/06	8 830 514		64	Didanosine, Stavudine			
10	671110	M	1999/11/29	312 587	68	56	No ART			
	671110	M	2000/08/03		81		Didanosine, Stavudine	Nevirapine		
	671110	M	2000/08/07			52	Didanosine, Stavudine	Nevirapine		
11	531113	M	2002/03/25	267 874 174		48	No ART			
	531113	M	2002/04/17				No ART			
12	731105	F	2000/10/02	296 990	1	62	No ART			
	731105	F	2001/08/13				No ART			