Predictors of Diffuse Myocardial Fibrosis in HIV Infected Persons: A Multiparametric Cardiovascular Magnetic Resonance Study

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Declaration

I, *HADIL SAAD*, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements is indicated otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Abbreviations

AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
ARV	Antiretroviral
CAD	Coronary artery disease
CD4	Cluster of differentiation 4
CMR	Cardiovascular magnetic resonance
CVD	Cardiovascular disease
CVF	Collagen Volume Fraction
DMF	Diffuse myocardial fibrosis
ECM	Extracellular matrix
ECV	Extracellular volume
EMB	Endomyocardial biopsy
Gal-3	Galectin-3
HAART	Highly active antiretroviral therapy
HASTE	Half-Fourier Acquisition Single-shot Turbo spin Echo imaging
HIV	Human immunodeficiency virus
HIVAC	Human immunodeficiency virus associated cardiomyopathy
HF	Heart failure
hsCRP	high sensitivity C-reactive protein
HTN	Hypertension
IE	Infectious endocarditis
LGE	Late gadolinium enhancement
LV	Left ventricle/ventricular
LVMI	Left ventricular mass indexed
NRTIs	Nucleoside reverse transcriptase inhibitors
OI	Opportunistic infection
SA	South Africa
SSA	Sub-Saharan Africa
STIR	Short tau inversion recovery
TB	Tuberculosis/tubercular
HTN	Hypertension
T1	T1 relaxation times
T2	Time constant for the decay of transverse magnetization
TI	Inversion time

Abstract

With the advent of effective antiretroviral therapy (ART), human immunodeficiency virus (HIV) is now a chronic disease. With increasing survival, cardiovascular disease (CVD) in people living with HIV is increasing in the ART era, with a changing epidemiology that is now largely characterised by diastolic dysfunction. Our hypothesis was that ART would be associated with regression of myocardial fibrosis in HIV. Myocardial fibrosis is associated with an unfavourable outcome in many different clinical settings. In this study, we used cardiovascular magnetic resonance (CMR) measurements of extracellular volume fraction (ECV) and tissue characterisation to assess diffuse myocardial fibrosis and determine the effect of ART use on diffuse myocardial fibrosis in HIV infected individuals on ART compared to untreated HIV infected persons.

Forty-four asymptomatic individuals with no known CVD who were HIV infected were included and classified into two groups: 25 on ART for >1 year (60% male, mean age 40 ± 9 years) and 19 ART-naïve (37% male; mean age 36 ± 8 years). All patients underwent CMR on a 3T Siemens Skyra scanner. Imaging included cine, T2 weighted (STIR), native T1 and T2 mapping, late gadolinium imaging (LGE) and ECV imaging.

HIV infected patients not on ART had a median time from diagnosis to entry in the study of 9 months. Treated patients had been stable on ART for over 12 months. There was no difference in left ventricular volumes, mass and function between treated and untreated HIV-infected patients. We found that, while elevated in both groups, the native T1 values were lower in the treated group compared to untreated patients. Again, while elevated in both groups, the ECV was slightly lower in the treated group, this did not reach statistical significance. There was no correlation between native T1 value or ECV with either disease duration or nadir CD4 count.

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We conclude that in patients with HIV, diffuse myocardial fibrosis provides valuable insights into the pathophysiology of HIV associated CVD and mechanism of diastolic dysfunction. Importantly, in this study, with a short lead period on ART, ART was associated with regression of diffuse myocardial fibrosis, as assessed by native T1, but not by ECV. Larger prospective studies are needed with longer follow-up to assess the role of CMR in both risk stratification and in tracking disease progression in HIV.

Chapter 1: Introduction

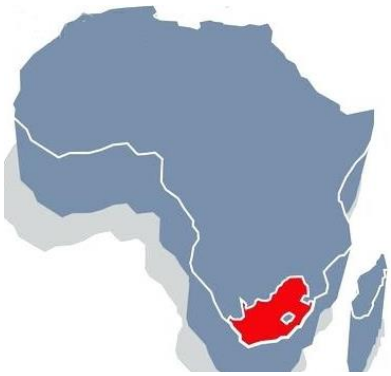
Since HIV/AIDS was first described amongst five individuals in the United States of America in 1981, it has become a global pandemic (1) . The region with the worst HIV/AIDS burden in the world is sub-Saharan Africa (SSA). With about 22.9 million people infected in 2011, SSA accounts for 69% of all people infected with HIV globally (2). Several biological, socio-economic, demographic and behavioural factors are known to increase the risk of HIV infection (3).

South Africa has the biggest and most high profile human immunodeficiency virus (HIV) epidemic in the world (see Fig. 1), with an estimated 7.1 million people living with HIV in 2016, and 56% of them on antiretroviral therapy (ART) (4). There were 270,000 new infections, while 110,000 South Africans died from acquired immunodeficiency syndrome (AIDS)-related illnesses in the same year (5). Representing a quarter of the estimated HIV infections in sub-Saharan Africa (SSA), the growing pandemic of HIV/AIDS is now the fourth leading cause of death worldwide (6).

South Africa has the largest ART programme globally, an effort that has been largely financed from its own domestic resources. The country now invests more than $1.5 billion annually to run its national HIV and AIDS programmes (7). Effective ART has dramatically reduced HIV/AIDS-related morbidity and mortality and decreased HIV transmission in the South African population (8).

HIV prevalence, however, remains high in the South African population, with an estimated prevalence of 19.2%, although this figure varies markedly between different regions (9). For example, the HIV prevalence is almost 40% in the Kwazulu-Natal province compared with 5 to 8% in the Western Cape and Northern Cape provinces, respectively (10).

The prognosis and long-term survival of people living with HIV/AIDS has improved significantly since the introduction of ART. However many people in Africa access ART late and commonly present with organ damage from HIV (11). Incidence of opportunistic infections (OIs) has also decreased in the ART era. Successfully treated HIV infected individuals remain at increased risk of a number of age-related non-AIDS morbidities, such as cardiovascular disease (CVD), cancer, and liver and kidney diseases (12). The incidence of diabetes mellitus has significantly risen with the availability and widespread use of ART, particularly in HIV patients receiving protease inhibitors (13). Long-term treatment with ART is associated with increases in both total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C), as well as reductions in high-density lipoprotein cholesterol (HDL-C) (14). As the survival of people living with HIV has increased, the incidence of HIV-associated CVD has also increased (15).



South Africa (2016)

7.1 million people living with HIV

18.9% adult HIV prevalence

270,000 new HIV infections

110,000 AIDs related deaths

56% adults on antirertroviral treatment

55% Children on antirertroviral

Fig.1.1Information of HIV and AIDS patients in South Africa, 2016(16)

Several studies have shown that HIV infection increases the risk of CVD (17). CVDs are currently a main cause of death among people living with HIV. HIV-associated CVD involves the entire cardiovascular tree and commonly affects all layers, including the myocardium, valves, pericardium, coronary, pulmonary, and peripheral vasculature.

The connection between HIV infection and CVD was established quite early in the history of the AIDS pandemic (18). Early studies in Africans with HIV/AIDS reported that CVD, involving predominantly the myocardium and pericardium, occurred in up to 60% of patients studied (19). The frequency and pattern of CVD in HIV-infected persons is determined by geography, access to ART and degree of immunosuppression (11). Several studies have reported the incidence of HIV-associated CVD to be much higher in SSA, compared to high-income countries (20).

The risk of CVD in HIV infected persons is influenced not only by the traditional cardiovascular risk factors, which are highly prevalent in this now ageing population, but also by genetics, family history, the effect of ART and the effect of HIV itself (21). Common HIV-associated CVD presentations in the Heart of Soweto Study included HIV-associated cardiomyopathy (38%), pericardial disease (13%) and pulmonary hypertension (8%) (22). The effects of HIV infection on the cardiovascular system have changed in post-ART era, with diastolic dysfunction being the predominant finding in the majority. Approximately 50% of asymptomatic HIV infected persons without known CVD have been reported from echocardiographic studies to have diastolic dysfunction (23). Studies from Africa have found the prevalence of diastolic dysfunction in HIV-infected patients to be much higher (86%) and to be more severe in patients with AIDS (24). At autopsy, 40% of HIV-infected patients were found to have histological evidence of interstitial fibrosis in historic studies [].

Despite effective suppression of viral replication, treated HIV infection is associated with persistent inflammation, tissue fibrosis, suboptimal immune recovery and organ damage(25). Early noninvasive detection of myocardial fibrosis is important; and evaluating the extent of

myocardial fibrosis may provide the ability to predict progression to ventricular dilation and adverse remodelling in patients with HIV. Myocardial fibrosis is an important part of cardiac remodelling that leads to diastolic dysfunction, heart failure and death (26). Myocardial fibrosis results from increased myofibroblast activity and excessive extracellular matrix deposition. Historically, the main method for detection of myocardial fibrosis has relied on histological evaluation of cardiac tissue specimens obtained from invasive endomyocardial biopsy (EMB). More recently, CMR has been demonstrated to be a safe, reliable and reproducible technique for the accurate assessment and quantification of focal and diffuse myocardial fibrosis(27).

Chapter 2: Literature review

Cardiac structure

The wall of the heart consists of three layers (Figure 2.1): the epicardium (external layer), the myocardium (middle layer) and the endocardium (inner layer). The epicardium is the thin, transparent outer layer of the wall and is composed of delicate connective tissue. The myocardium is comprised of cardiac muscle tissue. The thickness of the myocardium varies according to function and pressures that drive contractile force. The endocardium is a thin layer of overlying connective tissue, which provides a smooth lining for the chambers of the heart and covers the valves. The endocardium is continuous with the endothelial lining of the large blood vessels attached to the heart.

The pericardium is the membrane that surrounds and protects the heart; it is composed of two layers separated by a narrow cavity. The inner layer is firmly attached to the heart wall and is known as the visceral layer or epicardium. The outer layer is composed of relatively inelastic connective tissue and is termed the parietal layer. The pericardium is a fibrous layer that prevents distension of the heart, thus preventing excessive stretching of the heart muscle fibres. The cavity between the two layers of the pericardium contains a small volume of fluid which serves as a lubricant, facilitating the movement of the heart by minimising friction (28).

Cardiac muscle fibres are shorter in length and larger in diameter than skeletal muscle fibres. They also exhibit branching, which gives an individual fibre a Y-shaped appearance. Although cardiac muscle fibres branch and interconnect with each other, they form two separate functional syncytia, one for the atria and another for the ventricle

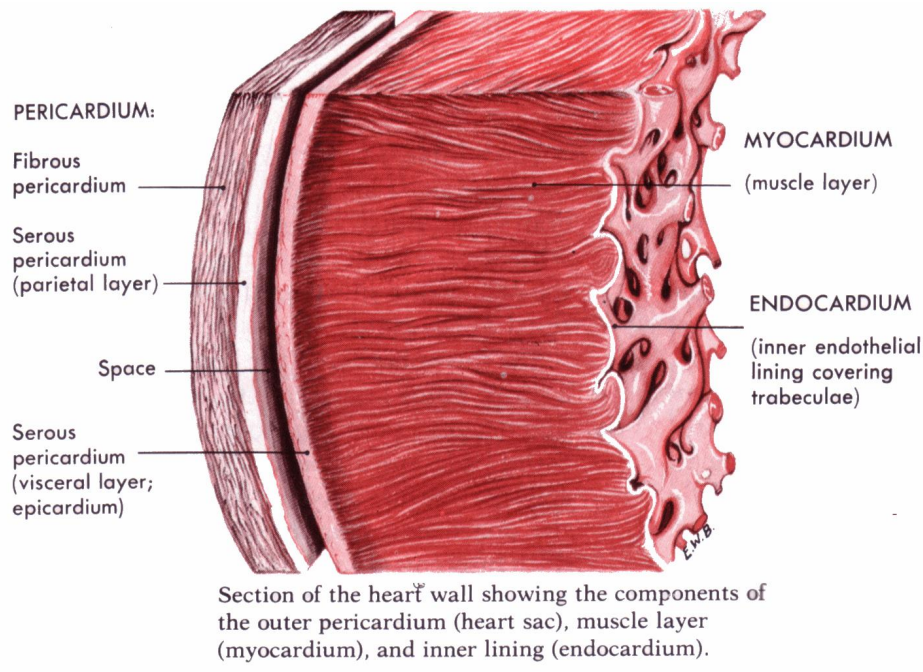


Figure 2.1 Layers Of The Heart, Histology Heart Wall (29), *http://histologyolm.stevegallik.org/node/347*

The normal myocardium includes a network of cardiac cells embedded within mainly fibril collagen. This collagen matrix plays an essential role in giving strength to the heart, as well as creating an intercellular communication grid. The myocardium can be characterised based on its cellular and extracellular or interstitial components:

- **Cellular components** include cardiac muscle (involuntary striated muscle fibres), which are interconnected by intercalated discs, structural components, nuclei, sarcolemma, sarcoplasmic reticulum, and vascular and neuronal elements (30).
- **Interstitial components** include the cardiac extracellular matrix, which is a complex network of fibres made from structural and nonstructural proteins, among which myocytes, fibroblasts, immune cells and vascular cells can be found. Collagen is the main structural protein, predominantly type I (80%) and type III (11%) collagen (31).

Collagen provides support and plasticity. In normal conditions, the collagen fibre network makes up only 2-4% of the cardiac structure. The cellular components of the myocardium are

embedded in the extracellular space that comprises fluid, collagen, elastin, fibrils, and other glycoproteins. The interstitium is a complex and dynamic environment, which is vital for normal cardiac structure and function. Interstitial extracellular space expansion is a distinctive feature of myocardial pathology and an important factor in ventricular remodelling.

Cardiovascular manifestations in HIV

HIV-associated cardiovascular disease (CVD) involves every segment of the heart and commonly affects all layers of the heart, including the myocardium, valves, pericardium and coronary, pulmonary, cerebrovascular, and peripheral vasculature. Clinical cardiovascular presentations associated with HIV infection include myocarditis, pericarditis, dilated cardiomyopathy (DCM), arrhythmias and vascular disease (11).

Myocardial disease

Cardiomyopathy

HIV-associated cardiomyopathy has evolved since its first description in the mid-1980s (32). HIV-associated cardiomyopathy was historically characterised by symptomatic, systolic dysfunction associated with a dilated LV that indicated a poor prognosis for HIV infected patients (33). In about 30% of patients with advanced immunosuppression, a dilated cardiomyopathy may still be observed, although the size of the heart chambers only slightly exceed the normal values and with a slight decrease of LV ejection fraction of about 45% (34). Systolic LV dysfunction has been replaced by subclinical diastolic dysfunction as the hallmark of HIV-associated cardiomyopathy in individuals with well-controlled HIV (35). In 'The Heart of Soweto Study' (Figure 2.2) the prevalence of HIV-associated cardiomyopathy was reported as 38%, comprising cardiac functions in both symptomatic and asymptomatic patients (22). Manifestations of HIV-associated cardiomyopathy include symptomatic heart failure with LV

dysfunction with or without concurrent LV dilation, any systolic impairment or diastolic dysfunction in asymptomatic HIV patients, and new onset heart failure in WHO stage IV HIV disease (22). In the course of cardiomyopathy, dilatation of the LV often coexists with mild myocardial hypertrophy (36), typically observed in the region of the posterior wall or interventricular septum.

There are several causes for HIV-associated cardiomyopathy (HIVAC) and clinical manifestations depend on the aetiology and the degree of host immunosuppression. Known causes include prior myocarditis, direct HIV toxicity, OIs, and nutritional deficiencies (37). While the prevalence of systolic dysfunction has decreased in HIV-associated cardiomyopathy, diastolic dysfunction is now seen in up to 64% of asymptomatic HIV-infected patients on ART (34). CMR studies report that these subclinical changes may be due, in part, to myocardial fibrosis and steatosis seen in patients on ART (38).

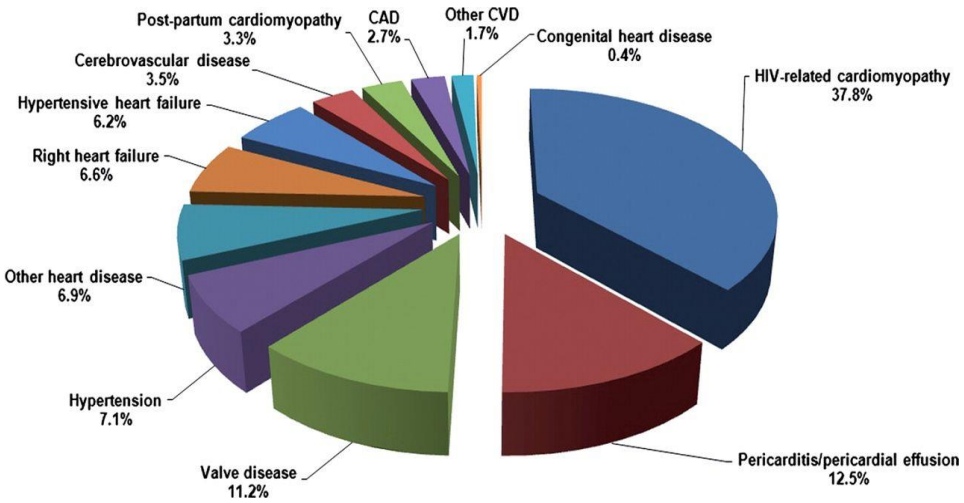


Figure 2.2 Primary cardiovascular diagnosis of all human immunodeficiency virus patients (%) presenting with De novo presentations of cardiovascular disease (CVD) in patients with HIV (n=518), Heart of Soweto Study (22).

Heart Failure

Heart failure, a common end-result of cardiac disease, appears to be more common among HIV patients (39). The incidence of HIV/AIDS-related heart failure is on the increase, and current evidence suggests that diastolic, rather than systolic, dysfunction is the predominant form of heart failure in the era of ART (23).

The pathophysiology of HIV-associated heart failure is multifactorial (Figure 2.3). Consequences of direct HIV infection, HIV components, drug toxicity, OIs, abnormal autoimmune responses to viral infection have all be implicated as causes of HIV-associated heart failure (40). HIV associated myocarditis, malignancy(41), myocardial fibrosis(42), endothelial dysfunction capillary leak syndrome and abnormal coagulation have also been considered in pathogenesis of heart failure in HIV (43). Also, traditional heart failure risk factors such as hypertension, diabetes, dyslipidaemia and smoking are more common in HIV infected persons (17).

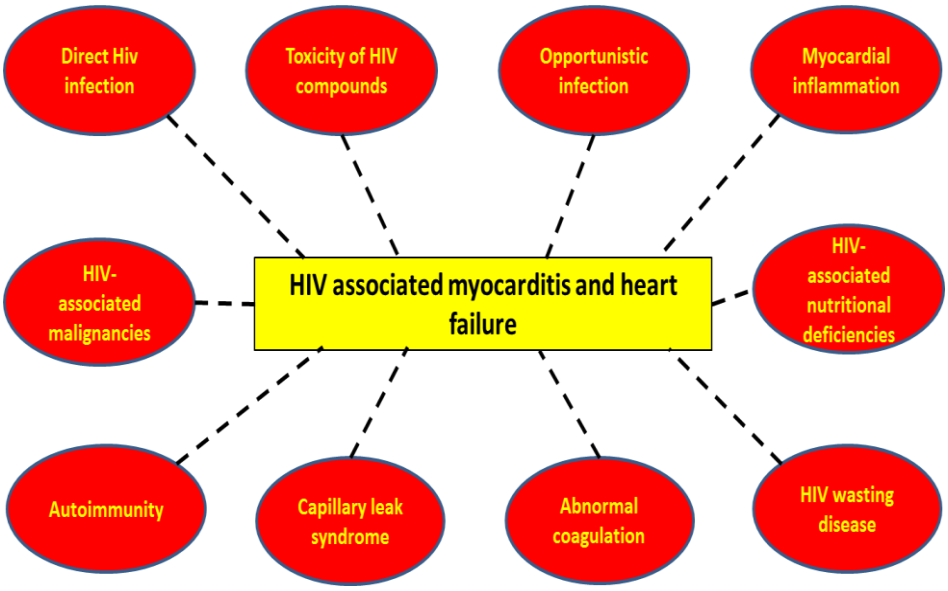


Figure 2.3 Causes of heart failure in HIV (44)

HIV infection is an independent risk factors for heart failure, particularly among patients without prior coronary artery disease (45). Early identification of subclinical changes in myocardial structure and function and their determinants can be particularly important in HIV patients (46). Recent studies have shown that even on ART, HIV infected patients remain at higher risk of heart failure (39). Further, CMR studies confirm involvement of myocardium and impairment of global and regional systolic function as well as diastolic function, even in asymptomatic HIV infected individuals on ART (38).

Myocarditis

Myocarditis is an inflammatory disease of the myocardium, defined by specific established histological, immunological, and immunohistochemical criteria based on EMB. Histology is the gold standard for establishing the diagnosis of myocarditis. Myocarditis can be produced by a variety of different causes, many of which are infectious, with viruses being a common cause of myocarditis. Common viral causes include enteroviruses (Coxsackie B and others), adenovirus, parvovirus B19, hepatitis C, and herpes virus 6. In LMICs, rheumatic carditis, Chagas disease, and conditions associated with advanced HIV are important causes of myocarditis. Myocarditis is histologically characterized by a lymphocytic infiltrate of the myocardium with necrosis and/or degeneration of adjacent myocytes not typical of the ischemic damage associated with coronary artery disease in subjects infected by HIV with or without evidence of OIs (47).

Direct invasion of cardiomyocytes by HIV has been described, but the virus affects the myocardial cells in a haphazard fashion, with no clear association between viral load and extent of myocardial involvement (48). However, the invasion of cardiomyocytes in HIV can be

through other microorganisms due to decreased immunity that makes them prone to infection. Typical infective causes of myocarditis may be fungal (Candida, *Histoplasma capsulatum* (49), *Cryptococcus neoformans* (50), Aspergillus), viral (*Herpes simplex* (51), cytomegalovirus (48), *Coxsackievirus B3* (52), Parvovirus (51)), bacterial, (*Mycobacterium tuberculosis*, *Mycobacterium avium*) (53), or parasitic (*Toxoplasma gondii*) (54).

"Myocarditis with lymphocytic infiltration has been reported to be present in 40–52% of patients who died of AIDS in the pre-ART era" (48). Various viral and OIs trigger myocarditis in the setting of uncontrolled HIV infection. Direct invasion of cardiac myocytes by cardiotropic viruses, including HIV, leads to a local cytokine release and subsequent infiltration of the myocardium with clonal expansion of B cells (55). Patients with confirmed myocarditis have an increased number of infected interstitial cells where proteolytic enzymes or increased concentrations of TNF-α or interleukin may injure the myocytes. Studies have revealed that these affected patients have increased concentrations of TNF-α, inducible nitric oxide synthase, and IL-6 (56). Reduction in OIs in patients on ART may be responsible for the impressive drop in myocarditis rates and declining prevalence of HIV-associated cardiomyopathy (57). Recently, CMR-based studies, including from our own group, reported that HIV infected persons had lower LV ejection fraction, higher LV mass, lower peak diastolic strain and strain rates and higher native T1 values (a measure of myocardial inflammation and fibrosis). Pericardial effusions and focal fibrosis on late gadolinium enhancement (LGE) CMR were more common in HIV infected persons (58).

Diastolic dysfunction

Diastolic dysfunction is one of the earliest manifestations of cardiovascular involvement in HIV infected persons (23). Diastolic dysfunction reflects an abnormality of the LV to relax

and/or to fill adequately with blood, at normal diastolic filling pressures (59). Diastolic dysfunction is considered as the first indication and an early marker of underlying cardiovascular disease in HIV uninfected patients without cardiac symptoms and preserved LV systolic function (60) . Diastolic dysfunction is the imaging hallmark of diffuse myocardial fibrosis. High levels of high sensitivity C-reactive protein (hsCRP), active tobacco smoking, and history of myocardial infarction were significantly associated with systolic dysfunction, while older age and hypertension were related to higher risk of diastolic dysfunction in HIV (46). Other studies have suggested that longer duration of HIV infection, higher body mass index (61), and exposure to zidovudine (62) were also associated with higher rates of diastolic dysfunction.

The clinical tool most frequently used to measure diastolic function is two-dimensional echocardiography, a noninvasive method of visualising cardiac structure and function. Echocardiography can accurately assess and grade diastolic dysfunction (63). Evaluation of diastolic function can also be accurately performed by CMR. CMR steady-state gradient echo can evaluate functional dimensions for time-volume curves; and myocardial tagging can assess ventricular diastole (63). In a cross-sectional study done on asymptomatic HIV infected patients, mostly on ART, there was a high prevalence of diastolic dysfunction (64). Diastolic dysfunction is present in up to 64% of asymptomatic HIV infected patients on ART in high-income countries (34).

The pathogenesis of HIV-associated diastolic dysfunction is likely multifactorial. Hypertension is associated with ART use, including prolonged duration of ART (65), and treatment with protease inhibitors (23). The increase in LV mass index is also associated with diastolic dysfunction (66). It is also possible that HIV or other associated viral infection will directly

affect the myocardium. Individuals with HIV infection have high rates of inflammation (23), which may predispose HIV infected patients to diastolic dysfunction. Patients with lower CD4 counts and longer duration of using NRTI have more common findings of diastolic dysfunction. NRTI use has previously been associated with cardiomyopathy and mitochondrial damage. Subclinical atherosclerosis as assessed by carotid artery intima-media thickness is common in HIV infected individuals (67). Finally, changes in glucose and lipid levels may also contribute to diastolic dysfunction. Several studies suggest that diastolic function is associated with HIV infection independently of ART (46). HIV infected ART naïve patients have reduced diastolic reserve that is not worsened by ART (68). These data reinforce the association of diastolic function with the HIV infection itself and its inflammatory response, and not with the ART use (68).

Myocardial fibrosis

Myocardial fibrosis is defined as a significant increase in the collagen volume fraction of myocardial tissue, which is common in advanced cardiac disease. The extracellular matrix (ECM) is an essential component of the healthy heart and functions to anchor cardiac muscle cells, regulate tissue mechanics, protects against myocardial rupture (69), provides a structural basis for the tight organisation of myocytes, prevents muscle fibre slippage and myocyte overstretching, and transmits contractile force and electrical signals (70). The ECM also has an important role in ventricular remodelling caused by altered conditions of preload or afterload (70).

An excessive accumulation of fibrosis, which leads to increased extracellular space, affects both the structural integrity of cardiac muscle and the electrical conductive properties of the myocardium (71). Normally, the ECM and fibrillar collagens network form only 6% and 2-

4%, respectively, of the structural space within the heart (72). An increased amount of collagen (fibrosis) in the myocardial tissue results from altered collagen turnover, in which net collagen deposition exceeds net collagen breakdown. Matrix metalloproteinases also play a key role in the development of myocardial fibrosis, which may be regionally distributed in the form of scar or be more diffuse, depending on the underlying cause (73). There are two types of myocardial fibrosis: replacement (macroscopic level) and interstitial fibrosis (microscopic level) (74). The former usually occurs as a result of myocyte necrosis after myocardial infarction. In fact, direct HIV infection of myocytes is rare (75). CMR studies have reported myocardial fibrosis in nearly all HIV infected patients studied (38). Focal myocardial fibrosis on CMR predominantly affects the basal inferolateral wall (38). In cross-sectional study of 95 HIV infected patients, CMR detected diffuse myocardial fibrosis of around 8%, and the authors postulated that cardiac fibrosis may be secondary to the downstream metabolic effects of HIV infection (42).

Pericardial disease

Pericardial disease can include asymptomatic pericardial effusion, pericarditis, cardiac tamponade, and constrictive pericarditis(76).

Pericardial effusion

Pericardial effusion is one of the most commonly identified CVD complications affecting HIV infected patients, developing in up to 20% of patients [102]. The prevalence of pericardial effusion before the ART era was 11% of patients with AIDS (79). After the introduction of ART, prevalence of pericardial effusion in HIV infected patients reduced by about 30%-35%, with a trend similar to that observed for HIV-associated cardiomyopathy (80). However, the majority of effusions were asymptomatic without an identifiable aetiology; those that were

symptomatic were likely caused by an identifiable infectious process or neoplasm (78). The effusions in HIV are usually part of a generalised serous effusive process that also includes the pleural and peritoneal surfaces (78).

Mortality of pericardial effusions in HIV infection is based on the severity and aetiology of the effusion, and usually higher when associated with tuberculosis (79). Most patients with a small pericardial effusion without tamponade are asymptomatic and do not require further testing other than follow-up, whereas pericardiocentesis is warranted for therapy in those with symptoms and to determine aetiology in patients with symptoms, even without tamponade (78). Pericardiocentesis has been found to be a safe and effective treatment of tuberculosis pericardial effusions in HIV infected patients (81). Pericardiocentesis is indicated for pericardial effusion when there are clinical signs of tamponade (such as elevated jugular venous pressure, dyspnoea, hypotension, persistent tachycardia, or pulsus paradoxus), or echocardiographic signs of tamponade (such as continuous-wave Doppler echocardiographic evidence of respiratory variation in valvular inflow, septal bounce, right ventricular diastolic collapse, and a large effusion) (82). HIV-infected patients with pericardial effusions generally have lower CD4 counts than those without effusion (83).

Pericarditis

At autopsy, pericarditis was found in 30% of AIDS patients (48). Pericarditis can be manifest in different forms: serous, fibrinous, serofibrinous purulent, or haemorrhagic (84). HIV-associated tuberculous pericarditis is considered an aggressive form with a massive degree of myocardial involvement. There are four recognised stages of tuberculous pericarditis and two general modes of clinical presentation. The stages include: a dry stage, an effusive stage, an adsorptive phase and a constrictive phase (85). In the first stage of presentation, pericardial

involvement is asymptomatic and is an incidental finding in patients who have evidence of active tuberculosis elsewhere in the body. In the second mode of presentation, inflammation and compression from inflammatory fluid, the diseased pericardium itself, or both, cause the typical constellation of symptoms that are associated with pericardial effusion and constrictive pericarditis (86). HIV may alter both the clinical manifestation of these recognised syndromes and the progression through the various stages (87). It is recommended that HIV should be considered as a diagnosis if a young patient presents with unexplained pericardial effusion or cardiac tamponade, particularly in SSA (88).

Tuberculous pericardial constriction in HIV

Constrictive pericarditis is characterised by constriction of the heart secondary to pericardial inflammation and fibrosis. Common causes include repeated pericarditis, previous cardiac surgery and radiation therapy (89). Tuberculosis is the main cause of constriction in SSA and in parts of Asia (90). Patients with tuberculous pericarditis often have concomitant HIV infection (91). Tuberculous pericarditis represents a form of extra-pulmonary tuberculosis. Other causes of constrictive pericarditis include neoplasms, autoimmune disorders and uraemia. Constriction causes impaired diastolic filling, in turn producing three important effects: (1) dissociation between intrathoracic and intracardiac pressures; (2) increased interventricular dependence – during inspiration, decreased LV filling induces increased RV filling, causing a right-to-left septal shift, and increases in the tricuspid inflow E velocity and hepatic vein diastolic forward velocity; and (3) increased cardiac filling pressures with pressure equalisation in all four cardiac chambers (89).

In the last two decades, the HIV pandemic has had a significant effect on the epidemiology of pulmonary and extrapulmonary tuberculosis. However, the impact of HIV infection on the

development of pericardial fibrosis in tuberculous pericarditis was uncertain (92). HIV reduces IL-13-secreting CD4+ TH2 cells, which regulate fibrogenesis directly, through stimulating collagen synthesis by fibroblasts and indirectly by promoting transforming growth factor beta-1 (TGF-β1) production by macrophages (93). Also, fewer granulomata have been observed in HIV infected tuberculous pericarditis patients with depleted CD4 lymphocytes, suggesting that there may be less propensity to develop pericardial fibrosis in HIV infected individuals with tuberculous pericardial effusion(94). There is some evidence that persons with HIV-associated tuberculous pericardial effusion may have a lower incidence of pericardial constriction than those with pericardial tuberculosis without HIV coinfection during 6 months of anti-tuberculosis chemotherapy(95).

Infective endocarditis in HIV

Infective endocarditis in patients with HIV/AIDS can be secondary to bacterial or nonbacterial (marantic) endocarditis. Infective endocarditis has been reported in adults with HIV infection, most commonly in intravenous drug users, specifically causing right-sided endocarditis (96). *Staphylococcus aureus*, *Streptococcus viridans* and Salmonella species are the most common organisms and the tricuspid valve is most involved valve (96). The prevalence of infective endocarditis in HIV infected patients is similar to that found in uninfected patients (48).

Nonbacterial (marantic) endocarditis is usually clinically silent, involves deposition of large, friable, sterile vegetations predominantly on the cardiac valves, and affects mainly the tricuspid valve which can lead to pulmonary embolization (97). Marantic endocarditis has been reported to occur in 3-5% of HIV infected patients, most commonly in those over 50 years of age (98).Neoplastic, hypercoagulable, and chronic wasting diseases have been linked with marantic endocarditis, which may lead to valvular dysfunction, most commonly left-sided lesions (78).

Patients with lower CD4 count, especially less than 200 cells/mm3, have a higher risk of endocarditis with much poorer prognosis (38).Treatment strategies for HIV infected patients with infective endocarditis are similar to those used for HIV uninfected patients. In the latter case, they are often part of a systemic OI with multiple organ localisation(99). Fungal endocarditis has been reported with increasing frequency as the AIDS epidemic has gained momentum, helped by the compromise of cell-mediated immunity in patients with HIV infection(84). Rates of infective endocarditis have decreased with the advent of ARV therapy(100). Symptoms associated with infective endocarditis include fever, chills, and dyspnoea, but can also include weight loss, concomitant pneumonia or meningitis(100). When intravenous drug use is excluded, HIV infection has not been shown to be a risk factor for infective endocarditis (101).

HIV-associated primary pulmonary hypertension

HIV infection can be complicated by pulmonary hypertension secondary to a combination of inflammatory, vascular and genetic factors(102). Idiopathic or primary pulmonary hypertension is a rare occurrence in HIV, and often carries a poor prognosis(103).The incidence of HIV-associated pulmonary hypertension has been estimated in 1/200, much higher than 1/200,000 found in the general population (104). Common histological findings include a plexogenic pulmonary arteriopathy similar to the findings in immunocompetent persons, a thrombotic pulmonary arteriopathy and a much rarer pulmonary veno-occlusive phenotype (105) .

HIV-associated primary pulmonary hypertension is more common in male and younger patients with risk factors including pulmonary infections, intravenous drug use, homosexual contacts, and haemophilia (16). However, pulmonary hypertension has been reported in HIV

patients without a history of thromboembolic disease, intravenous drug use, or pulmonary infections (106). Although multifactorial and poorly understood, the pathologic mechanism has been described to be partly the result of endothelial damage and vasoconstriction due to HIV-induced release of endothelin-1, IL-6, and TNF-α, as well as secretion of TNF-α, oxide anions, and proteolytic enzymes by alveolar macrophages in response the infection (107). Normal endothelial structure is replaced by plexogenic pulmonary arteriopathy, which is characterised by remodelling of the pulmonary vasculature with intimal fibrosis (83).

Pulmonary hypertension carries a poor prognosis in HIV-infected patients: in a single prospective comparison, although HIV infected patients with pulmonary arterial hypertension were younger and had a lower disease severity than their noninfected counterparts, investigators found similar rates of mortality between groups (108). Two recent studies found that CD4 count was independently associated with survival in 154 patients with HIV and pulmonary arterial hypertension, with pulmonary hypertension as the direct cause of death in 72% of those affected (106). Investigations done on 47 HIV-associated pulmonary arterial hypertension patients within the Swiss Cohort Study reported that patients receiving ART had a significantly decreased median RV systolic pressure over right atrial pressure gradient (-21 mmHg) as compared with patients who did not receive ART (+25 mmHg) (109). Exercise tolerance in pulmonary arterial hypertension is commonly assessed by means of the 6-minute walking distance (6MWD). Recently, long term ART improved only the 6MWD, but not haemodynamic parameters, in HIV-associated pulmonary arterial hypertension without specific treatments (110). Therapy for pulmonary arterial hypertension in HIV is the same as in HIV uninfected persons. Anticoagulation may be considered on the basis of individual risk-benefit analysis (83). Pulmonary hypertension (PAH) identified with HIV can happen in any phase of the disease where HIV-infected patients have a 2500-fold increased risk of developing

PAH when compared with noninflected individuals. Mortality is high, even in the era of antiretroviral treatment (ART)(111).

HIV-associated coronary artery disease

HIV-infected patients are known to be at risk for premature coronary artery disease (CAD) (112)(113). Different factors related to HIV can lead to development atherosclerosis, including immune dysfunction, proliferation of T-cells, inflammation, endothelial dysfunction, and lipid abnormalities (114,115). During atherogenesis, HIV promotes monocyte penetration of the vascular intima to promote secretion of cytokines and expression of endothelial cell adhesion molecules (116). The process of endothelial dysfunction in HIV patients may be driven by HIV transcription factors (117). Increased risk of CVD in HIV infected patients is directly related to lower CD4+ T-cell counts (118). Higher number of activated CD8+ T-cells is observed in relation to increased rates of coronary artery plaque and carotid artery stiffness (119)(117). Increased frequency of carotid artery plaques was reported in one study in participants with CD4+ counts< 200 cells/mL (120). Also, increased levels of proinflammatory cytokines in HIV (high-sensitivity C reactive protein, IL-6, D-dimer, and cystatin C) suggest that inflammatory and prothrombotic changes may lead to atherogenesis, subsequent plaque rupture and endothelial dysfunction (121).

Changes happen in lipids levels in HIV patients leading to a further increased risk of CAD. In the early stage of HIV infection both total cholesterol and high-density lipoprotein cholesterol are decreased (122). Also, lower levels of apolipoprotein B and smaller low-density lipoprotein (LDL) cholesterol have been reported in more advanced stages (123). In addition, deleterious metabolic effects such as dyslipidaemia and insulin resistance after exposure to certain ART treatments have been reported (114). Recent studies observed that HIV infected patients

presented with large thrombus burden than atherosclerotic plaques suggesting *de novo* arteriothrombosis and thrombophilia as possible causes of CAD events (124).

Cardiovascular effects of ART treatment

South Africa has the largest ART programme in the world (Figure 2.4). In 2017, more than 56% of people living with HIV in the country were receiving ART, compared to 2012 when just 31% of people living with HIV were on ART (7). First-line regimens need to be simple and manageable for patients to stay on lifelong therapy and to avoid unnecessary and costly switching or substitution of drugs. Second- and third-line ART regimens are far more expensive than the first-line, and it is important to prolong the durability of first-line ART as far as possible.

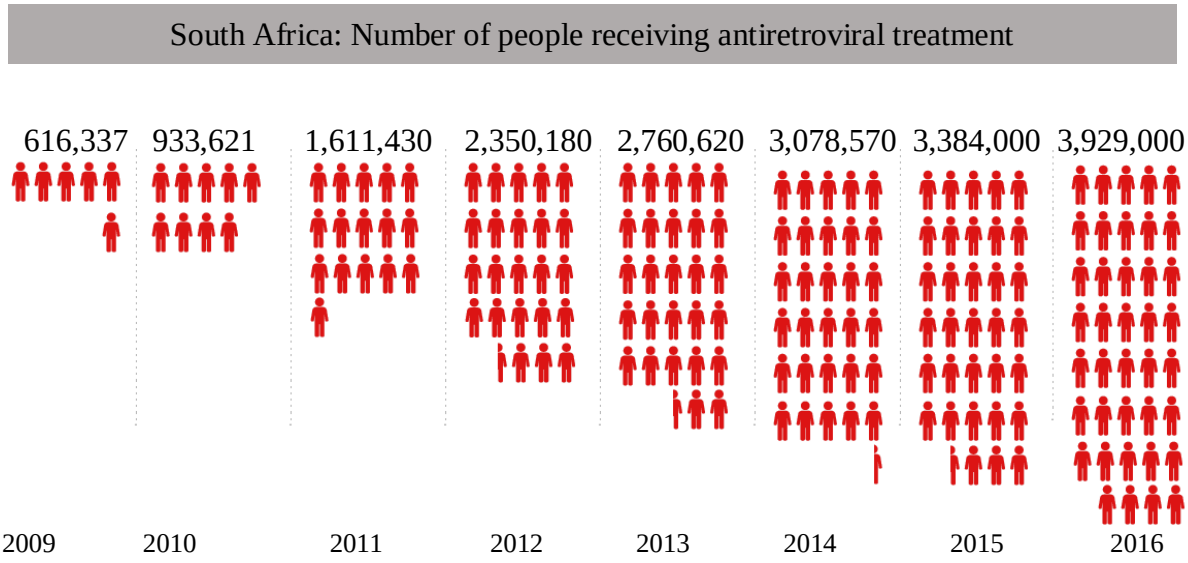


Figure 2.4 Number of ART receiver in South Africa in recent years from 2009 to 2016

South African activists and health care workers have helped to drive down the exorbitant cost of treatment. Since 2004, South Africa has issued three sets of ART guidelines (125). ART has transformed the clinical profile of HIV from an acute infection with a high mortality into a

treatable, chronic disease (15). Since the widespread initiation of ART the prevalence of HIV-associated CVD has dropped by 30% in this region (126).

Exposure to protease inhibitors increased the relative rate of myocardial infarction by 16% per year (127). Exposure to abacavir and tenofovir is associated with an increased risk of cardiovascular events and heart failure (128). Diastolic dysfunction is also common in long-term survivors of HIV infection. ART adverse effects include ART-associated dyslipidaemia and insulin resistance, which may contribute to an increased risk of cardiovascular events (129). HIV and ART have direct effects on adipose tissue and the liver, with subsequent dyslipidaemia, lipodystrophy and insulin resistance. Other direct effects include endothelial and vascular dysfunction leading to hypertension, atherosclerosis and myocardial infarction (130). In adults with ART-related fat redistribution, several studies have suggested an increase in the risk of myocardial infarction relating to the level of viral control or to ART exposure (131). ART induces increases in cholesterol, triglycerides, and LDL-cholesterol.

Chapter 3: Myocardial fibrosis

The myocardium is a complicated structure of several cell types: some of them are resident cells (i.e. cardiomyocytes, fibroblasts, endothelial cells and smooth muscle cells), while others are migratory cells (lymphocytes, plasma cells, mast cells and macrophages). Migratory cells interact with permanent cells in both pathological and physiological conditions to support cardiac functions (132). In pathological conditions, in the myocardial interstitium there is a population of cells called myofibroblasts that are extensively involved, together with fibroblasts, in synthesis and excessive deposition of ECM (133). Diffuse interstitial fibrosis results from progressive increase in collagen synthesis by myofibroblasts. The increase in collagen volume fraction leads to diastolic dysfunction primarily, and later may be associated with ventricular dilatation and systolic dysfunction (134). Furthermore, increasing myocardial fibrosis has been shown to correlate with progressive deterioration of myocardial function (135). Diffuse myocardial fibrosis in animal and human studies is associated with worsening LV systolic function, abnormal cardiac remodelling, and increased ventricular stiffness (136).

Types of cardiac fibrosis

Myocardial infarction (necrosis) results in sudden loss of a large number of cardiomyocytes leading to replacement fibrosis (Figure 3.1, panel A). Interstitial fibrosis is associated with increased deposition of collagen in the cardiac interstitial space in the absence of significant cardiomyocyte loss (Figure 3.1, panel B). Perivascular fibrosis is characterised by expansion of the vascular adventitial matrix (Figure 3.1, panel C). The fibrotic heart exhibits expansion of the interstitial space associated with deposition of collagens and other matrix proteins (Figure 3.1, panel D). Myofibroblasts are the main effector cells in cardiac fibrosis; however, macrophages, lymphocytes, mast cells, vascular endothelial cells and cardiomyocytes may also

participate in the process (71). The types of cardiac fibrosis have been summarized in branch layout in Fig. 3.2.

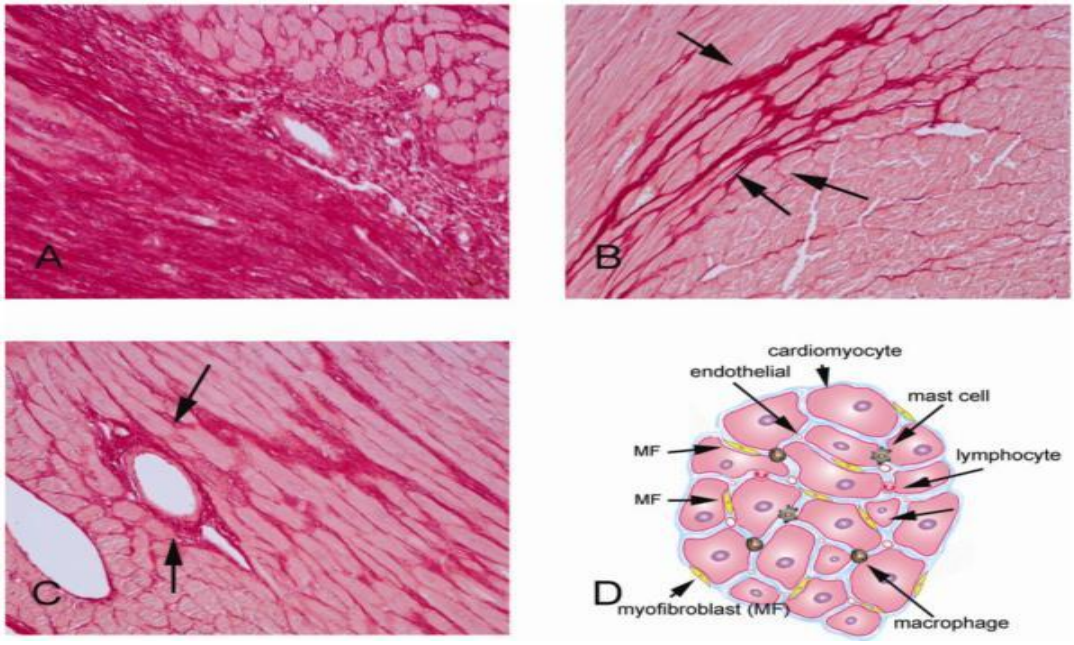


Figure 3.1 Histological structure of fibrotic myocardium, (A) replacement fibrosis, (B) Interstitial fibrosis, (C) Perivascular fibrosis, (D) fibrotic heart (137)

Replacement myocardial fibrosis

Replacement fibrosis is the accumulation of collagen corresponding to necrotic or damaged myocytes. It is characterised by the macroscopic distribution of fibrosis in the myocardium that is caused by the replacement of damaged or necrotic cells by plexiform fibrosis and is only seen when the integrity of the cell wall is affected. Both regional and diffuse patterns of replacement fibrosis may be present, depending on the underlying cause, can occur because of myocyte necrosis after acute myocardial infarction (138). Myocyte death triggers the activation of cardiac fibroblasts, leading to an inflammatory response that governs myocardial collagen turnover and subsequent extracellular fibre deposition (139). In nonischaemic conditions, recurring toxic insults might lead to cellular apoptosis and induce inflammatory responses, resulting in localized formation of collagenous scars. Replacement fibrosis may have a

localised distribution as in ischaemic LV dysfunction, myocarditis, hypertrophic cardiomyopathy, sarcoidosis) or a diffuse distribution as in chronic renal insufficiency, toxic cardiomyopathies and miscellaneous inflammatory disease (140) . LGE CMR is a validated, noninvasive way to identify replacement fibrosis.

Interstitial myocardial fibrosis

Interstitial fibrosis, Fig. 3.2, is diffusely distributed collagen in the extracellular space. In general, it is diffuse due to a balance between myocyte growth and death. Interstitial fibrosis also governs the process of progressive age-related fibrotic remodelling (141). Disruption of this equilibrium by conditions such as hypertension, pressure and volume overload events, diabetes mellitus, and obesity leads to increased interstitial fibrosis. Its subtypes include reactive and infiltrative fibrosis (142).

Reactive fibrosis

This type of fibrosis has a progressive onset and present in a variety of common conditions, including aging, hypertension, diabetes, acute or chronic ischaemia (without infarction), valvular disorders, dilated cardiomyopathy, (143) transplant rejection, hypertrophic cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, sarcoidosis, systemic lupus erythematosus, systemic sclerosis, Chagas disease, and chronic renal insufficiency (144). It is caused by an increase in collagen production and deposition by stimulated myofibroblasts, from alterations in metabolism, activation of the renin-aldosterone-angiotensin system, and oxidative injury and results in diffuse collagen deposition in the myocardial interstitium (138).

Infiltrative fibrosis

This type of fibrosis is much rarer and is caused by progressive deposition of insoluble proteins or glycosphingolipids in the interstitium. Examples of infiltrative fibrosis include amyloidosis and Anderson-Fabry disease (145).

Eventually, both interstitial and infiltrative fibrosis lead to cardiomyocyte apoptosis and replacement fibrosis (Figure 3.2) (138).

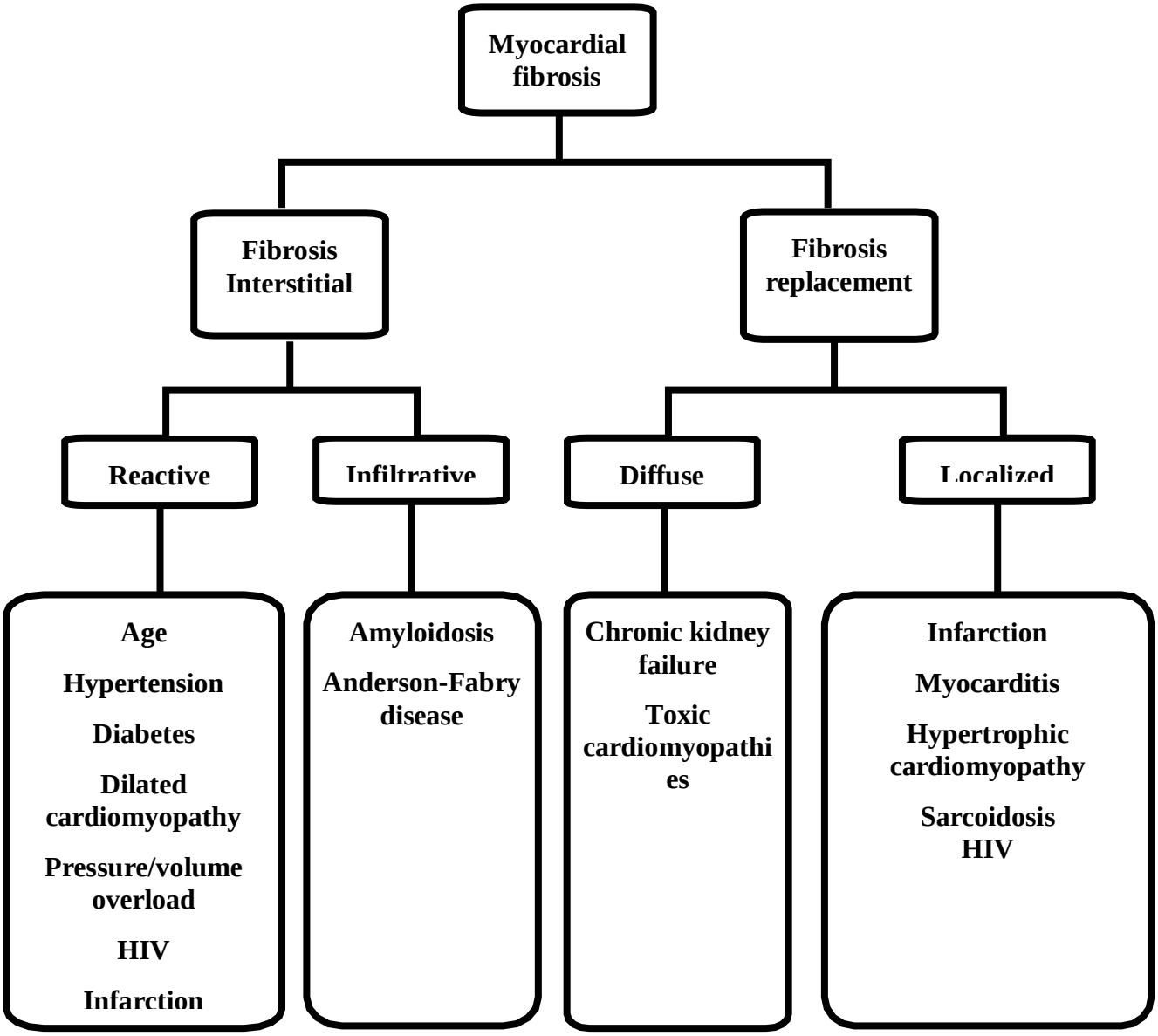


Fig 3.2. Sub-types of myocardial fibrosis and main causes

Detection of Myocardial Fibrosis

Fibrosis is associated with the pathological remodelling of the LV and heart failure progression (146). Early detection of fibrosis would be beneficial in diagnosis, following progression of CVD, and intervening to improve survival in CVD patients. Myocardial fibrosis manifests as QRS prolongation (147), frequent ventricular premature beats, and ventricular tachycardia (VT) on electrocardiogram. Echocardiography may detect increased LV end diastolic diameter, decreased LV ejection fraction, and elevated LV filling pressures, with abnormal Doppler indices. Diffuse myocardial fibrosis may lead to impaired movement of the entire ventricular wall. Presently, effective evaluation and detection of fibrosis includes use of EMB, CMR and serum biomarkers of collagen turnover (148).

Serum markers

In recent years, galectin-3 and soluble ST2 have emerged as robust myocardial fibrosis biomarkers (149). Galectin-3 is a reproducible marker for the detection of early cardiac remodelling and is secreted by inflammatory cells and fibroblasts. Circulating levels of Galectin-3 are associated with the degree of myocardial fibrosis (150). ST2, a member of the interleukin-1 receptor family, exists in both a transmembrane and a soluble form. Interleukin-33 (IL-33) plays a role in suppressing myocardial fibrosis and cardiomyocyte hypertrophy via binding to the transmembrane ST2, whereas excessive soluble ST2 can attenuate the function of IL-33, leading to myocardial fibrosis and ventricular dysfunction. Many studies indicate that ST2 is associated with all-cause mortality and cardiovascular mortality (151).

MicroRNAs (miRNAs) are a cluster of short RNAs that regulate increased expression of proteins involved in collagen synthesis. miRNAs respond to ECM deposition and expansion

by promoting either the degradation or the translational repression of their target mRNAs, while playing a vital role in myocardial fibrosis regulation (152)(12).

Other serum biomarkers associated with myocardial fibrosis, as measured by histological parameters and collagen volume fraction (CVF), are the carboxy-terminal pro-peptide of pro-collagen type I and the amino-terminal pro-peptide of pro-collagen type III. The disadvantage of the use of these two biomarkers is their low specificity for cardiac fibrosis (153).

Endomyocardial biopsy

Previously, the only gold standard methodology available to assess myocardial fibrosis was histopathology assessment of EMB or autopsy specimens. The methodology enables qualitative macroscopic assessment after Masson's trichrome staining (154), and quantitative absolute assessment of the CVF. The CVF is the ratio of the sum of all connective tissue areas over the sum of all connective tissue and muscle areas from averaged values of several representative fields of the tissue section; and can detect the extent of myocardial interstitial fibrosis (155). EMB has many disadvantages and risks as invasive biopsies may not detect regional myocardial fibrosis (156). In clinical practice, according to moral and ethical considerations, the use of EMB is not routine and not effectively applied.

Cardiovascular magnetic resonance

CMR is a noninvasive diagnostic tool that plays a key role in the assessment of cardiac morphology and function, and provides a simple means of detecting myocardial fibrosis in a variety of pathologies. CMR may be helpful in identifying and characterising myocardial abnormalities and in assessing the effects of HIV on the myocardium. Two CMR techniques are useful for detection of fibrosis: (1) LGE CMR and (2) T1 mapping.

Late gadolinium enhancement CMR

LGE is a well-established CMR technique widely used to evaluate ischaemic and nonischaemic myocardial diseases (157). LGE can be used for accurate quantification and visualisation of patterns of dense focal ECM deposition as seen in replacement fibrosis. The assessment of myocardial fibrosis is best performed after injection of gadolinium contrast agent that reduces the T1 relaxation time of myocardial tissue (74). Visualisation of fibrosis by CMR is based on a greater distribution volume and slower washout of gadolinium contrast agents within tissue with greater extracellular space due to oedema or fibrosis (158). Enhancement in CMR due to myocardial fibrosis causes the enlargement of the extracellular space because of ECM deposition and excessive retention of gadolinium. LGE imaging depicts the relative difference in longitudinal recovery (T1) times between enhancing areas of fibrosis or scar (T1 shortened due to accumulation of extracellular gadolinium contrast agent) and normal nulled myocardium (longer T1 as gadolinium contrast agent is more rapidly washed out) (159).

The increase in gadolinium concentration within fibrotic tissue causes T1 shortening, which appears as bright signal intensity in the CMR image. Different patterns of enhancement have been reported, depending on the underlying aetiology (160) . For instance, in ischaemic myocardium, the enhancement is either in the subendocardium or transmural, while in nonischaemic myocardium, it can be in subendocardium, mid-myocardium, epicardium or global in the following diseases: idiopathic dilated cardiomyopathy, myocarditis, hypertrophic hearts, right ventricular pressure overload, sarcoidosis and Anderson-Fabry, Chagas disease, amyloidosis, systemic sclerosis and post cardiac transplantation (161).

LGE has several limitations. First, it is not a quantitative technique: signal intensity is measured using arbitrary units. Second the LGE myocardial fibrotic tissue is defined based on the difference in signal intensity between fibrotic and normal myocardium and this difference

generates the image contrast. So, LGE is incapable of absolute quantification of myocardial scar and assessment of diffuse interstitial myocardial fibrosis. These limitations are overcome by the myocardial T1 mapping, which enables direct signal quantification and characterisation of myocardial tissue on a standardised scale (74).

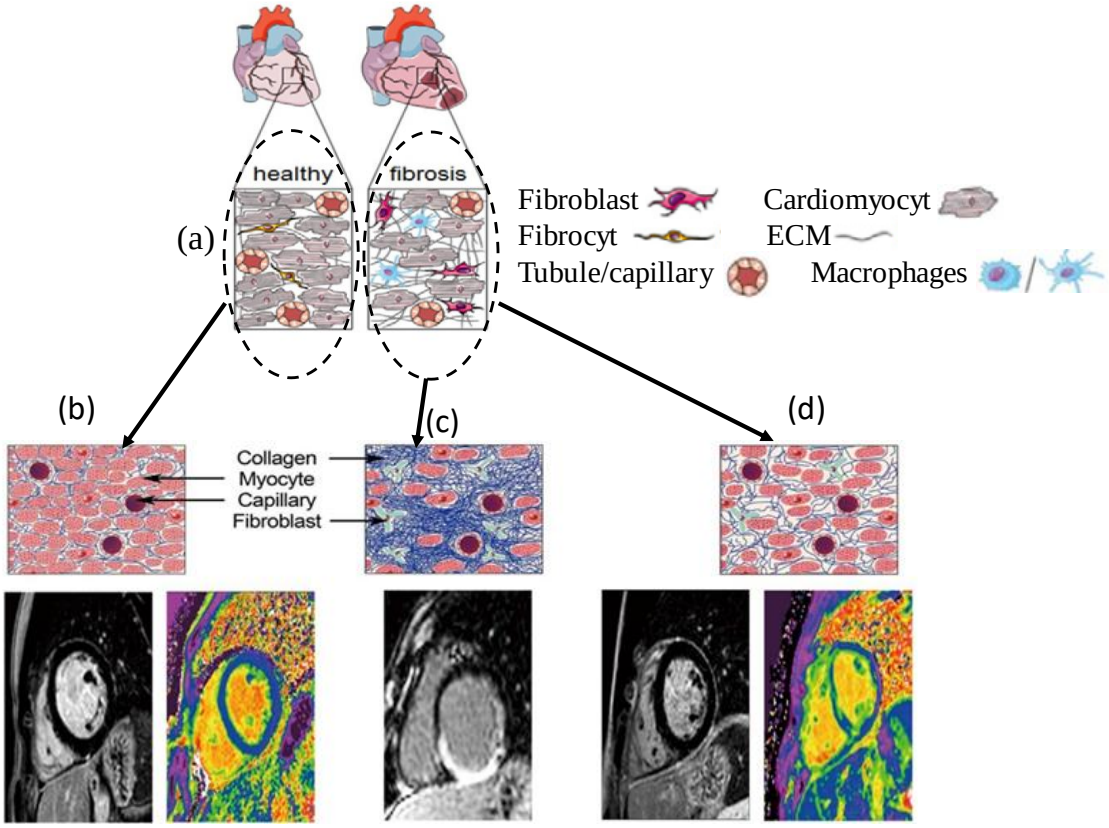


Figure 3.2(a) Pathological characteristics of fibrosis in heart tissues. In tissues, common events lead to fibrosis progression (and regression). If the pathological stimulus is persistent and the healing process is dysregulated, the continuous recruitment and activation of inflammatory cells and myofibroblasts can result in fibrosis. Core features of fibrotic processes that are shared by all these tissues include overproduction of cytokines, growth factors, ECM proteins and ultimately the loss of architecture as well as function. Histological normal cardiac tissue (b) and subtypes of fibrosis; Replacement fibrosis(c) Reactive interstitial fibrosis (d) and correlation with CMR(162).

T1 Mapping

T1 mapping has been recognised as the elective noninvasive method to quantify diffuse myocardial fibrosis. T1 mapping, by means of the multi breath-hold technique, is one of the most commonly utilised methods for T1 quantification (163). It provides parametric maps representing T1 relaxation times values (in milliseconds) on a voxel-by-voxel basis. A T1 map is a two-dimensional slice image where each voxel of the image displays the T1 relaxation time as signal intensity using a colour scheme for easier visual assessment. High T1 relaxation times are observed in diffuse fibrosis, protein deposition, and interstitial water as in oedema (164). Low T1 values are seen in iron or lipid deposition (165). T1 relaxation time is based on cellular and interstitial components of the myocardium. Native myocardium with ischaemic scar shows longer T1 values compared with unaffected remote myocardium. After contrast administration, regional and diffusely scarred myocardium shows longer T1 relaxation and delayed normalisation of T1 times with gadolinium washout. Whereas these observations show the potential of T1 mapping for the evaluation of myocardium fibrosis, native and postcontrast T1 values provide indexes with high diagnostic accuracy for the discrimination of normal and diffusely diseased myocardium. T1 mapping allows for the accurate quantitation of diffuse and infiltrative interstitial fibrosis (166). Normal ranges reported for native myocardial T1 values at 1.5 T using the MOLLI sequence are from 930 to 990 msec (167). Native T1 mapping is more useful in the case of ECM expansion induced by diffuse fibrosis (e.g. interstitial fibrosis in *hypertrophic cardiomyopathy* and *dilated cardiomyopathy*), than by focal fibrosis (replacement fibrosis, infarction scar) (168).

ECV quantification and ECV mapping

ECV is considered as a marker of myocardial tissue remodelling and provides a physiologically intuitive unit of estimation. Standard gadolinium-based contrast agents are appropriated all through the extracellular space and shorten T1 relaxation times of myocardium proportional to the local concentration of gadolinium [2]. Regions of fibrosis and scar will hence show shorter T1 relaxation times, in particular after contrast administration.

The ECV technique introduces a potentially important new method to examine the myocardium because it is sensitive to the distribution within the LV myocardium of cellular and extracellular interstitial (ECM) compartments. Alterations in these compartments occur from different physiologic and pathophysiologic biologic processes (138). The interstitial space can be assessed directly using standard gadolinium chelates but these low-molecular weight, purely extracellular agents are small enough to pass across the vascular wall into the extracellular space, yet are large enough not to be able to penetrate cells with intact membranes. They accumulate passively in the gaps between cells through post-bolus tracer kinetics and the increased ECV of interstitial expansion in "scar" tissue (169). The ECV of the myocardium reflects the volume fraction of heart tissue that is not taken by cells. ECV maps can also be generated on a pixel-wise basis if native and post-contrast T1 images are co-registered, quantified, and adjusted for the haematocrit (170). Expansion of the myocardial ECV represents a nonspecific increase in free water in the myocardium and occurs in a variety of pathologies, including focal and diffuse fibrosis, oedema, and amyloidosis. In the absence of amyloid or oedema (171), expansion of the myocardial collagen volume fraction is responsible for most ECM expansion which culminates in mechanical, electrical and vasomotor dysfunction. Fibrosis is associated with a few conditions and is considered to represent a final common pathway of myocardial disease from a variety of insults.

As published recently, ECV correlated with the degree of LV myocardial fibrosis percentage only in patients without inflammation, not in those with inflammation. In cases of inflammation, ECV correlated with results from T2 mapping. ECV correlated significantly with fibrosis in the T2 ≤ 59 ms but less so in those with T2 > 59 ms (172). This is of relevance because ECV does not measure CVF itself but is affected by extracellular oedema and hyperaemia, both of which are seen in myocardial inflammation.

Hypotheses and aim

Aim

The main aim of this project was to investigate the role of ART on modulating diffuse myocardial fibrosis in HIV infected patients, by assessing differences in native T1 and ECV between ART-treated and untreated HIV infected patients.

Hypotheses

The first hypothesis for this study was that diffuse myocardial fibrosis would be more severe in ART naïve patients and that the use of ART would be associated with regression in diffuse myocardial fibrosis in HIV-infected patients.

A second hypothesis of this study was that diffuse myocardial fibrosis in HIV would be related to duration of HIV infection and nadir CD4 count.

Chapter 4: Materials and Methods

Ethics

Local institutional ethics committee (University of Cape Town Human Ethics Research Committee and Groote Schuur Hospital Research Ethics Committee) approval was obtained for the studies in this project, as they involved human subjects. All study participants gave written informed consent prior to participation.

Patient population

A total of 44 patients (22 males, average age of 39 ± 9 years, average heart rate of 82 ±17 beats per minute) were examined under the approved protocol. Patients were recruited from the general medical wards and outpatient clinics at Groote Schuur Hospital. There were two study groups:

Group 1: HIV infected patients receiving ART (n = 25 cases)

Group 2: HIV infected patients not receiving ART (n = 19 cases)

Inclusion and exclusion criteria

The inclusion criteria were:

- A confirmed diagnosis of HIV infection
- Age ≥18 years
- Treated group must have been on ART for ≥1 years
- Untreated group were ART naïve

The exclusion criteria were:

- Contraindications to MRI
- Glomerular filtration rate <30ml/min
- ALT>2x the upper limit of normal
- Non-sinus rhythm
- Claustrophobia in patients

Study design

The study was a cross-sectional study, with patients in the two groups assessed at a single time point.

All research was conducted at Groote Schuur Hospital, Cape Town, South Africa. Subjects collected from hospital inpatients and from infectious diseases clinic and were assessed for suitability to undergo CMR prospectively. All subjects, included in this study, should not have any cardiac problems previously and during our study. Additionally, those with previous clinical CMR imaging were invited to participate. For the evaluation of differences between two subjects groups with and without ART, subjects with ART should be under treatment for not less than one year. There were two components to this study.

The first part was assessed cardiac fibrosis as with CMR, utilizing novel tissue characterization software. The second part was to investigate the relationship between ART treatment and myocardial fibrosis. Informed consent was obtained from all participants, and the study was conducted under the guidelines of the Groote Schuur Hospital Ethics Committee.

Cardiovascular magnetic resonance

Magnetic resonance imaging (MRI) is one of the important technological advances of the 21st century. CMR is a robust and reproducible technique, which is noninvasive and does not involve any ionising radiation. It has very high spatial and temporal resolution and has become

a complementary imaging modality for several cardiovascular conditions. Moreover, CMR is the gold standard technique for assessment of biventricular structure and function (e.g. volumes, ejection fraction and wall motion abnormalities), myocardial mass, and myocardial viability. CMR plays a crucial role within the morphologic and functional assessment of CVD. High spatiotemporal resolution, inherent contrast resolution, wide field of view (FOV) and multiplanar imaging capabilities make CMR an ideal tool for the investigation of cardiovascular diseases.

Basic of physics of CMR

A nucleus with odd number of protons and/or odd number of neutrons possess angular magnetic moment, and can create an electromagnetic field, as discovered by Bloch (173). Such nuclei are referred to as "spins", and their magnetic moment are represented by a magnetisation vector for convenience (Figure. 5.1). The hydrogen nucleus has a single proton and is most frequently imaged in MRI because of abundant water in the human body. Other popular atoms include ^{31}P, ^{31}C, and ^{19}F which are useful for studies on cellular metabolism. MR images are formed based on the interactions between “spins” and three external magnetic field components: a strong static field (B0), a radiofrequency (RF) field ($B1_t$), and linear gradient fields (G_t). (174)

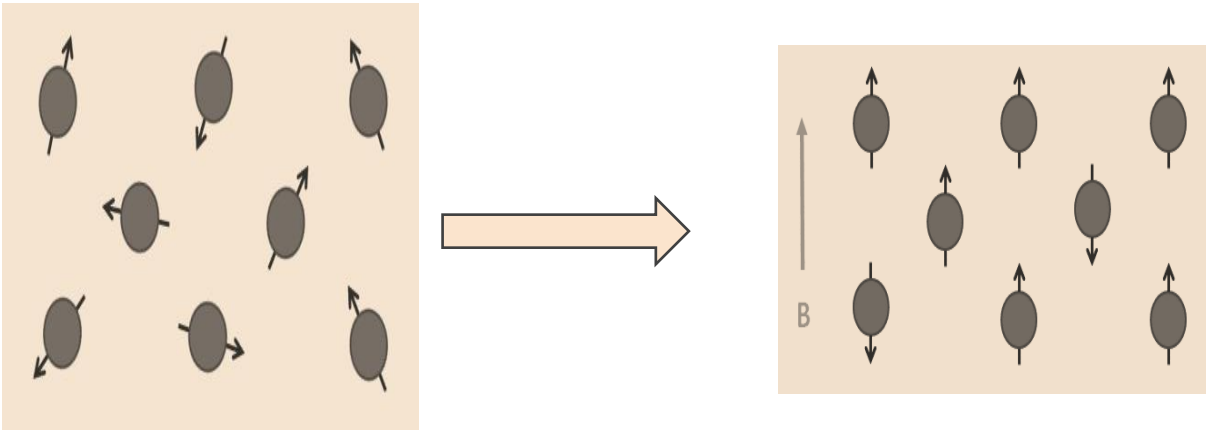
In the absence of static field (B0), the axes of spin magnetic moments are arranged in a random way so that their net moment is zero. However, when an external magnetic field (B1) is applied, they align either with (parallel) or against (antiparallel) the external field. The preferred state of alignment is the one that requires the least energy: that is, parallel to B0. Accordingly, more protons align with B0 than against it. The difference in the number of protons aligning parallel and antiparallel to B0 is typically very small but ultimately depends on the strength of B0 as

well as the temperature of the sample. The difference in spins aligned in the two directions will create net magnetic moment, which is called polarisation (Figure 5.1).

The polarisation phenomenon is like the alignment of a compass needle by the earth's magnetic field. Each magnetic spin not only spins around its own magnetisation axis, but also precesses around the axis of B_0. The speed of precession, that is, how many times the protons precess per second, is measured as the precession frequency (also named the Larmor frequency, ω_0, in MHz) and determined by the Larmor equation:

$$\omega_0 = \gamma\beta_0 \quad (1)$$

Where γ is a constant for a nuclear species (e.g. hydrogen) termed the gyromagnetic ratio. Its value for the proton is 42.6 MHz/T. The Larmor equation indicates that precession frequency is proportional to the strength of the magnetic field.



Absence of static field B0

Presences of static field B0

Figure 5.1 Nuclear spins with and without a static field (175).
https://wikidoc.org/index.php/Basic_MRI_Physics

In CMR, the pixel signal intensity is based on the relaxation of hydrogen nuclei protons in the static magnetic field, of typically 1.5 or 3.0 Tesla scanners. The relaxation of the hydrogen

nucleus proton is specifically characterised by 3 distinct MR parameters: (1) the longitudinal relaxation time (T1) or spin-lattice relaxation time that corresponds to the specific time decay constant when the proton recovers 63% approximately of its longitudinal magnetisation equilibrium value; (2) the transverse relaxation time (T2) or spin-spin relaxation time that corresponds to the specific time when the proton transverse magnetisation drops to approximately 37% of its original value; and (3) , proton density (PD), which explains the pixel signal intensity as the density of mobile hydrogen atoms within the tissue voxel or proton density. Both of those T1 and T2 times are measured in milliseconds.

Both T1 and T2 relaxation times depend on the molecular environment of the water molecules in the tissue and therefore characterise each tissue specifically. T1 and T2 relaxation times vary significantly from one type of tissue to another, but also within the same tissue depending on its physiopathological status (e.g., inflammation, oedema, and fibrosis). The CMR techniques used will also result in different contrast images. Specific CMR sequences can be used to selectively reveal certain molecular environments within the tissue. Those differences are further enhanced with the use of gadolinium extracellular magnetic resonance contrast agents.

The CMR protocol

Patients were recruited for enrolment into the study. Informed consent was obtained from all patients. Patients underwent CMR imaging at CUBIC UCT centre on a 3T Skyra (Siemens, Erlangen). A large 18-channel flex coil was placed over the abdomen and chest and used simultaneously with the 32-channel spine array coil. After standardised patient-specific planning, a stack of breath-hold short axis balanced steady-state free precession cine slices covering the LV was acquired for quantification of volume, mass and EF.

The CMR protocol was as follows (Figure 5.2):

1. Localizers
2. Haste
3. Long axis and short axis cines
4. T1&T2 weighted imaging
5. Cine tagging
6. LGE
7. Postcontrast T1 mapping

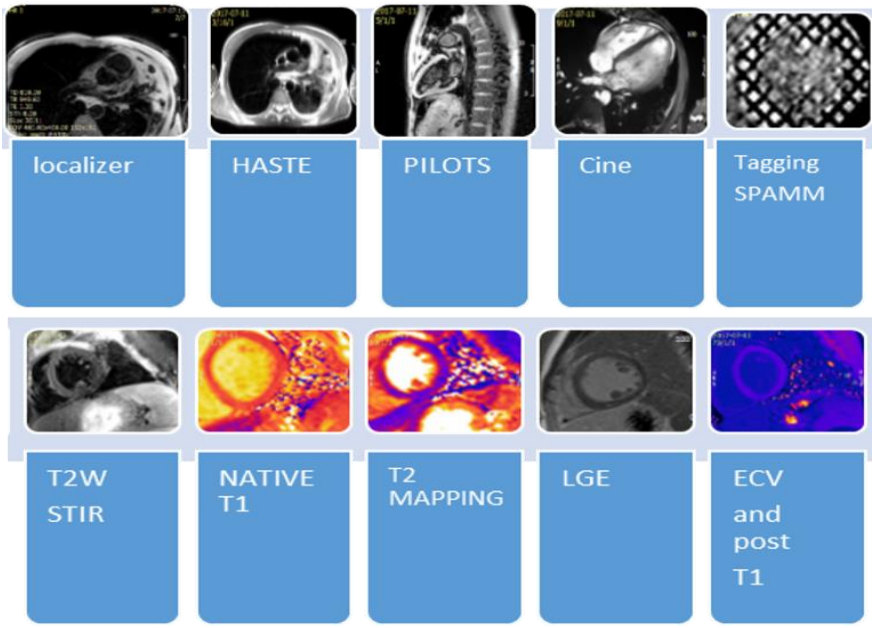


Figure 5.2 Different CMR sequences used and analysed

For post processing analysis of the images, the following software programs were utilised:

1. CVI42

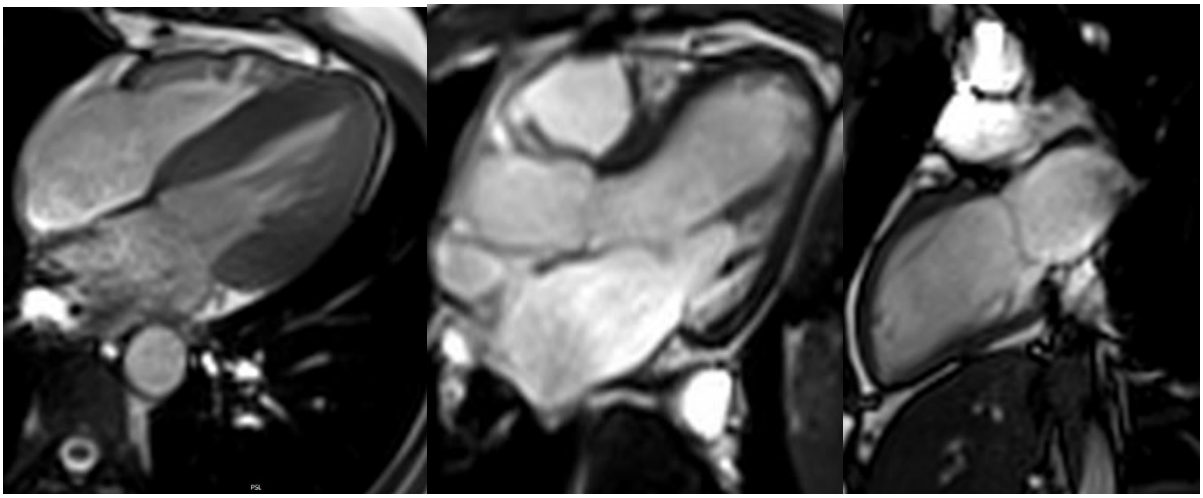
2. Siemens ARGUS VE11D

Analysis of CMR images

Cine images

Imaging parameter were as follows: balanced steady state free precession (bSSFP) cine imaging (Figure 5.3) in short- and long-axis views with typical parameters: acquisition matrix = 139×208, repetition time = 45 ms, echo time = 1.67 ms, flip angle = 44^{O}, voxel size = 1.7 × 1.7×8 mm^3, slice thickness = 8 mm, inter-slice gap = 1.2mm.

The sequence protocol was prefaced by survey images in coronal, sagittal, and transverse orientations. Retrospectively gated steady-state free precession cine images were acquired in vertical long-axis (VLA), horizontal long-axis (HLA), and short-axis (SA) orientations at 3 Tesla (Figure 5.3). Analysis of LV and RV functions was performed using CVI42 software.



(a) (b) (c)

Figure 5.3 Cines images of (a) four chamber, (b) three chamber and (c) two-chamber views.

Using a segmented approach, data was acquired over 10 – 15 heart beats to build up 20 images throughout the cardiac cycle at a single imaging plane. Therefore, the acquisition of each imaging slice required a 5 to 15 second breath-hold to avoid respiratory motion artefacts. Ten

to fifteen short axis imaging slices were normally acquired to cover the entire LV. An entire stack of wall motion images took approximately 10 minutes to acquire, allowing for some rest time between successive breath-holds. The spatial resolution of cine MRI was typically on the order of 1.7x 1.7 x 8 mm^3, which was adequate to detect abnormalities in the thickening of the myocardial wall.

LV short axis epicardial and endocardial borders were manually contoured at end-diastole and end-systole. LV end systolic (LVESV) and end diastolic (LVEDV) volumes were used to calculate stroke volume (SV) and ejection fraction (EF): (EF = SV/EDV). Myocardial mass was also calculated by subtracting the endocardial volume from the epicardial volume, based on prior knowledge of myocardial specific gravity. Same to right ventricle (RV) but only contouring endocardium (see Figure. 5.4).

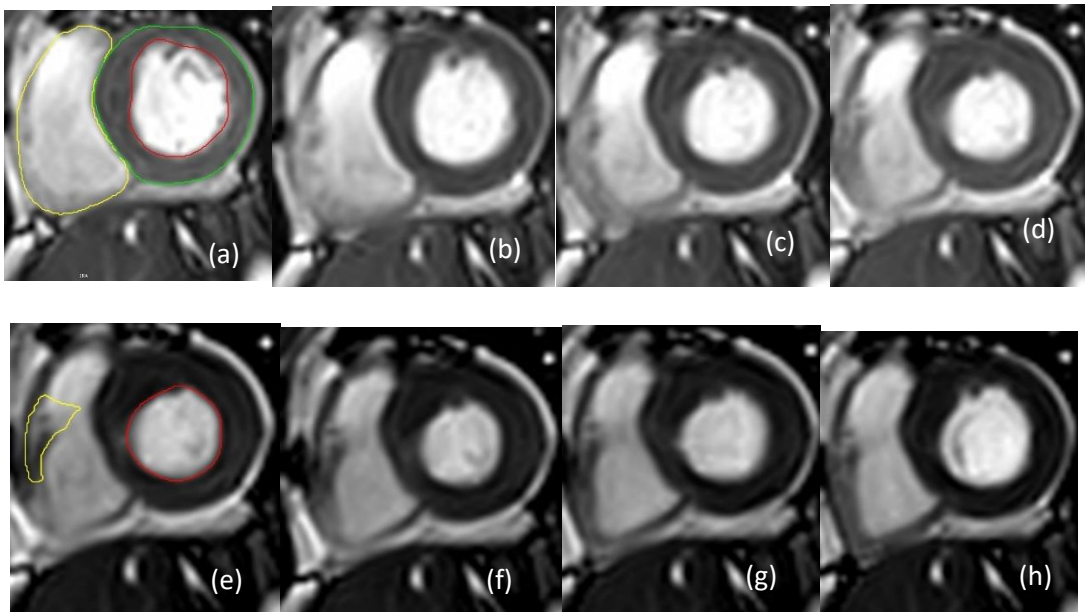


Figure 5.4 Images of a human left ventricle acquired using CRI. Shown are 8 (a-h) of the 20 acquired images at different phases of the heart cycle of a single short-axis slice. Each image is acquired at an effective temporal resolution of 50 ms. Image (a) shows the heart at end-diastole, while image (e) shows the heart at end-systole.

The endocardial (inner) and epicardial (outer) contours drawn on the end-diastolic and end-systolic frames define the varying thickness of the LV wall, and are used to calculate the ejection fraction and the LV volumes and mass.

T1- and T2-weighted images

Quantitative analysis was performed by comparing the LV myocardium in short axis against adjacent skeletal muscle in the same slice in T1&T2 weighted, verified on a corresponding balanced steady state free precession (SSFP) image. The signal intensity (SI) ratio was defined as (SI myocardium/SI remote skeletal muscle). Myocardial oedema was diagnosed when myocardial T2 SI ratio was >1.9. Care was taken to exclude nonsuppressed blood pool signal due to slow flow adjacent to the sub endocardium and to avoid using areas with abnormally low signal for normalisation.

LGE images

Patients received a total of 0.2 mmol of gadolinium diethylene triamine penta-acetic acid (Gd-DTPA) per kilogram of body weight intravenously. The LGE images were acquired with a T1-weighted segmented inversion recovery turbo fast low-angle shot sequence with the following parameters: acquisition matrix =140 × 256, inversion time individually chosen on TI scout, repetition time = 750 ms, echo time = 1.96 ms, flip angle = 20°, pixel or voxel size = 1.4 × 1.4×8 mm^3 , slice thickness = 8 mm, inter-slice gap = 1.2 mm; after a 6-minute delay of administration intravenous administration of gadolinium . The inversion time (T1) was adjusted for optimal nulling of normal myocardium. Usually, gadolinium contrast agents reduce the T1 relaxation time of adjacent tissue. Based upon various specific properties of the tissue the T1 shortening induced by the gadolinium will generate specific differences in signal intensity.

Images of the LV (whole short axis slice from base to apex were acquired at increasing inversion times (TI) starting at 300 ms and increasing to 400-450 ms for the final image. At short inversion times, image acquisition was quick, and multiple images could be obtained in a single breath-hold. With increasing TIs, duration of image acquisition increased, limiting data acquisition in a single breath hold. Images were evaluated qualitatively for the presence or absence, pattern (subendocardial, mid-wall, subepicardial, and transmural) and regional distribution of LGE areas. Quantitative analysis was performed by manually contoured LV epicardial and endocardial to draw (ROI) and comparing normal myocardium to evaluate volume fraction of LGE (Figure 5.5).

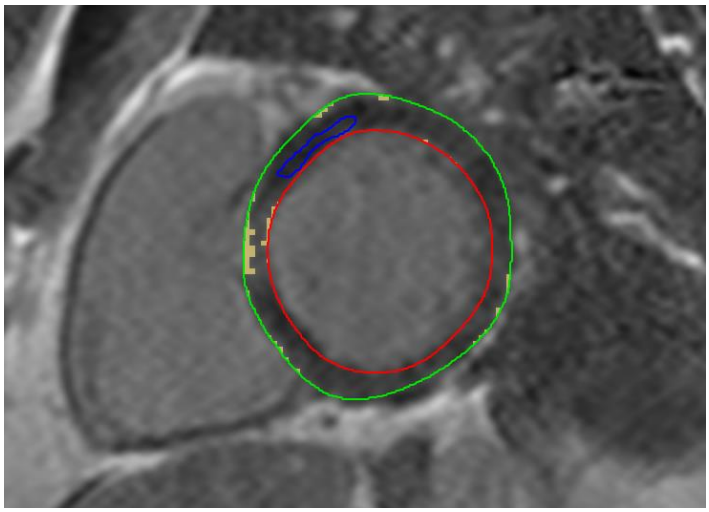


Figure 5.5 Quantification of LGE volume fraction for region of interest

T1 mapping

The technical protocol for T1 mapping requires a series of images using different inversion times to derive a T1 recovery curve resulting in a map that describes the relaxation value on a pixel-by-pixel basis. Multiple technical approaches are currently available to obtain a T1 mapping study, all of them derived from an electrocardiographically gated inversion-recovery

(IR) sequence, including the Look-Locker (LL) sequence, the modified LL inversion-recovery (MOLLI) sequence (176), and the shortened MOLLI (ShMOLLI) sequence (177). Recently, other sequences for T1 mapping were introduced such as saturation recovery single-shot acquisition (SASHA) and saturation pulse prepared heart-rate-independent inversion recovery (SAPHIRE) (178). In this study, we used the MOLLI sequence which allows accurate and reproducible *in vivo* measurement and T1 mapping of myocardium with high spatial resolution within a single breath-hold. T1 mapping (including native T1, postcontrast T1, and ECV) and has ability to accurately quantify of diffuse and infiltrative interstitial fibrosis, where the apparent increase in interstitial space is not observable using LGE, owing to its lack of sensitivity to detect this size and pattern of fibrosis.

Native T1

The native T1 is obtained without administration of contrast material and reflects a composite of both intra- and extracellular compartments which shows cellular and extracellular portions of the myocardium (179). Noncontrast native T1 relaxation time is emerging as a viable alternative to gadolinium contrast use when gadolinium contraindicated because of renal impairment (180). The native T1 value can change in processes such as intracellular lipid accumulation, iron overload, and interstitial fibrosis and oedema (181) . One of the advantages of this modality is that is can be performed safely on patients with advanced kidney failure and pregnant women. Myocardial native T1 times have been shown to be significantly higher in haemodialysis patients compared to control subjects (182). Native T1 has similar ability for predicting histological CVF as ECV (183). Many factors affect native T1 values, including the field strength used (e.g., 3T versus 1.5T) and pulse sequence used to estimate T1 (e.g., MOLLI versus ShMOLLI) (184).

Postcontrast T1 mapping and extracellular volume (ECV) fraction

T1 mapping performed after administration of gadolinium-based contrast medium, shortens T1 relaxation times and reveals myocardial properties mostly at the cellular level. Although providing increased signal, these postcontrast T1 values need to be corrected for a range of factors including T2 time, individual variation in gadolinium kinetics and time from contrast medium administration to imaging. Gadolinium concentration has a strong nonlinear relationship with the R1 relaxation rate (1/T1) and measuring the change in T1 in both the myocardium and blood pool following contrast medium administration allows the concentration of gadolinium in these compartments to be estimated.

The ratio of myocardial contrast medium concentration to blood concentration is termed the partition coefficient (λ) and corrects for many of the above confounders. At contrast equilibrium, the gadolinium concentration will be equal in the myocardium and blood pool. Knowing the blood volume of distribution (1-haematocrit) allows the myocardial volume of distribution to be calculated as a surrogate for the extracellular space, defined as the ECV. Normally, cardiac ECM is a complex network of fibres made from structural and nonstructural proteins, among which myocytes, fibroblasts, immune cells and vascular cells can be found. ECV is defined as a coefficient of the changes in T1 in tissue and blood before and after contrast injection.

The absolute fibrosis volume can then be calculated by multiplying the ECV by the end-diastolic volume (185). Postcontrast T1 mapping is used for mostly calculating the ECV fraction in combination with native T1. ECV mapping has been used for quantification of both focal and diffuse myocardial fibrosis (186). The advantage of this type of methodology is that

it minimises systematic errors in technique, enables better comparison of scans at different time points.

Partition coefficient (*λ)*	ΔR1myo/ΔR1blood
ECV	(1−haematocrit) · (ΔR1myo/ΔR1blood).
Fibrosis volume	ECV×(LV MASS/Myocardial density)

ΔR1= (1/postcontrast T1-1/precontrast T1)

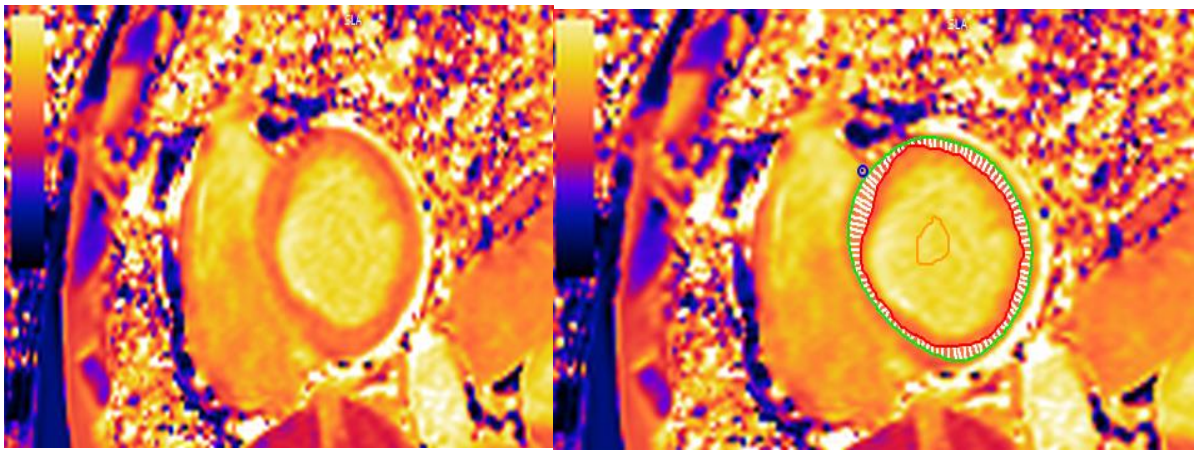
Myocardial density = 1.05 g/ml (187)

There are several factors that influence the specific T1 values measured during scanning, including the image acquisition protocol, postprocessing, scanner magnetic field strength (T), contrast medium dose, and time delay to postcontrast imaging. Normal ECV values of 25.3 ± 3.5% [1.5 T] have been reported in healthy individuals (188).

T1 mapping in this study was obtained with a Modified Look-Locker Inversion recovery (MOLLI) with a 5(3)3 scheme in 3 single slices. Short axis orientation (base, mid and apex) before (T1 native) and 15 min after intravenous contrast agent injection with the following parameters: acquisition matrix = 144 × 256, echo time = 1.12 ms, repetition time = 280.56 ms, flip angle = 35°, pixel size = 1.41 × 1.41mm^2, slice thickness = 8 mm. Quantitative analysis was performed using native T1 and LGE T1 (postcontrast T1) images by manually contoured (ROI) area and blood pool area. The estimation of ECV was calculated by used the equation:

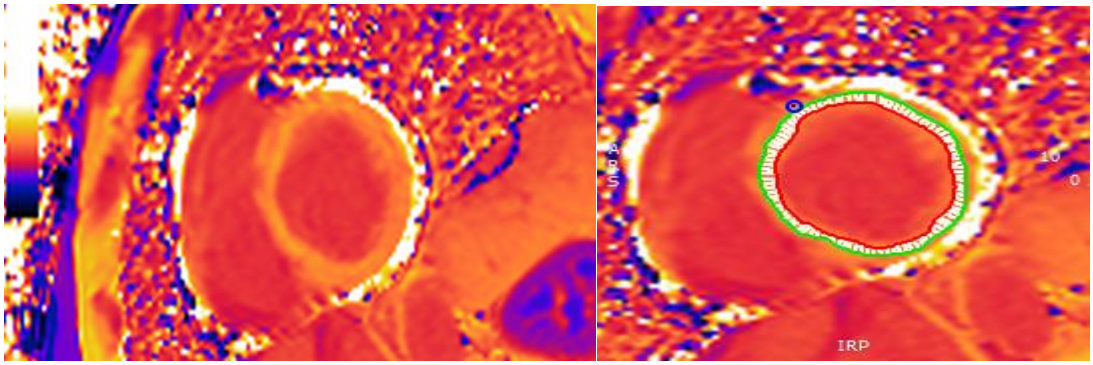
$$ECV = (1 - \text{haematocrit}) \cdot \left(\frac{\frac{1}{post-contrast\ T1\ myocardium} - \frac{1}{pre-contrast\ T1\ myocardium}}{\frac{1}{post-contrast\ T1\ blood} - \frac{1}{pre-contrast\ T1\ blood}} \right) \quad (2)$$

A region of interest (ROI) in the septum was chosen for estimation of noncontrast T1 ($T1^{native}$), postcontrast T1 ($T1^{contrast}$) and ECV, as previously described (189). Care was taken to avoid the endocardium/blood pool interface. If regional enhancement was seen in the septum on the LGE image a septal ROI was chosen adjacent to the enhanced region for T1 mapping analysis (Figures 5.6 and 5.7).



(a) (b)

Figure 5.6 Contouring of native T1(a) before contouring (b) contouring native T1 by using cvi 42 software with value 1210±20



(a) (b)

Figure 5.7 Post contrast T1 mapping (a) before contouring (b) after contouring by use cvi 42 with value 615±10

T2 mapping

T2 mapping, as shown in Figure 5.8, was performed before contrast injection in a single slice, free-breathing, navigator-gated, multi-echo sequence in basal, mid-LV and apical short-axis slices using a 3-point T2-prepared bSSFP sequence with the following CMR parameters: acquisition matrix = 116×192, echo time = 1.32 ms, repetition time = 207.37ms, flip angle = 12°, pixel size = 1.9 × 1.9mm^2, slice thickness = 8 mm.

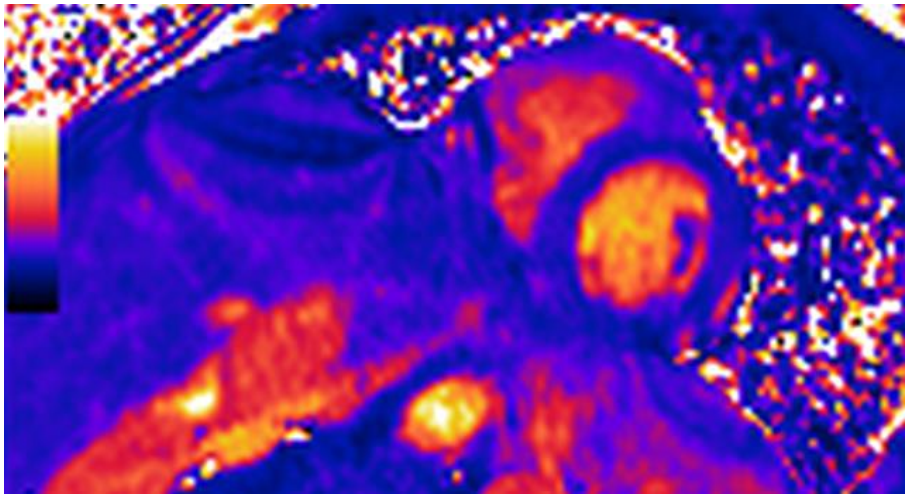


Figure 5.8 CMR T2 mapping image by use cvi 42 with value of 39±4

Data statistical analysis and reporting

The data was either binary/categorical and some measured on a continuous scale. Summary statistics were reported for each CMR assessment variable for group 1 and 2. Normality of data was tested using the Kolmogorov-Smirnov test. Normally distributed data was presented as mean ± standard deviation (SD) or, where highly skewed, as median (interquartile range). Non-parametric data was presented as numbers (percentages). The *chi-square* test or the Mann-Whitney U test was utilised for non-parametric data. Unpaired samples between groups was assessed by the unpaired 2-tailed Student t test. Correlation was assessed using the Pearson "R" and Spearman "RS" coefficient, as appropriate. All statistical tests were two-tailed, with p-values of less than 0.05 considered statistically significant. All analysis was performed using STATA version 12.1 (Lakeway Drive, College Station, Texas 77845 USA).

Chapter 5: Results

In this study we aimed to study the effect of ART on diffuse myocardial fibrosis in HIV infection. A total of 44 patients who were HIV infected were evaluated. There were 25 patients not on ART, and 19 who were receiving ART. The clinical characteristics of the study population, from the 44 patients, are summarised in Table 1.

There were no differences in age, sex, body mass index heart rate, haematocrit, renal function and duration of HIV disease between HIV infected patient on ART and those not receiving ART. All patients in both groups had no previous history of heart disease.

Table 1: Demographic characteristics separated by HIV infection status

	HIV infected on ART (n=25)	**HIV infected not on ART (n=19)**	***P*-value**
Age, years	40± 9	36±8	0.13
Male / Female	15 / 10	7 / 12	0.68
BMI, kg/m^2	24±7	27±8	0.29
Heart rate	80±15	84±19	0.44
Haematocrit, %	34±6	32±7	0.35
Creatinine, µmol/l	74±17	85±60	0.34
CD4 account cells/µl	337±341	132±121	
Duration of disease before ART commencement, months	9±5	N/A	–

Values are mean ± SD or n (%). *p*-values of the direct comparison between HIV naïve and HIV on ART are shown in the last column, using Mann-Whitney U, BMI: body-mass-index, SD Standard deviation.

Table 2: CMR imaging parameters for left and right ventricular geometry and LGE

	HIV infected on ART (*n* = 25)	**HIV infected not on ART (*n* = 19)**	***P*-value**
LV			
LVEDV, ml	141±50	136±39	0.705
LVEDV index, ml/m^2	79±25	74±19	0.461
LVESV, ml	67 (49-68)	61 (41-68)	0.532
LVESV index, ml/m^2	38(28 -41)	33 (23 -40)	0.380
LVSV, ml	74±23	75±21	0.798
LV Mass, g	106±28	103±30	0.724
LV Mass index, ml/m^2	59±14	56±14	0.389
LVEF, %	54±11	56±8	0.492
RV			
RVEDV , ml	123±35	124±36	0.873
RVESV , ml	67±24	64±21	0.731
RVEF, %	46±9	48±6	0.420
LGE			
no of patients with enhancement	n=17	n=17	
LGE pattern	Mid-wall, patchy &linear	Mid-wall, patchy &linear	-
Volume fraction LGE, %	33±9	32±10	0.933

LV left ventricular, EDV end-diastolic volume, ESV end-systolic volume, EF ejection fraction.

Table 3 CMR Mapping Parameters (native T1, T1 contrast, ECV, T2)

	HIV infected on ART (*n* = 25)	**HIV infected not on ART (*n* = 19)**	***P*-value**
-T1 native, ms	1259±46	1300±58	<0.011
-T1 contrast	646±61	651±65	0.771
-ECV	31±5	32±5	0.596
-T2, ms	40±4	42±4	0.159

ECV extracellular volume fraction.

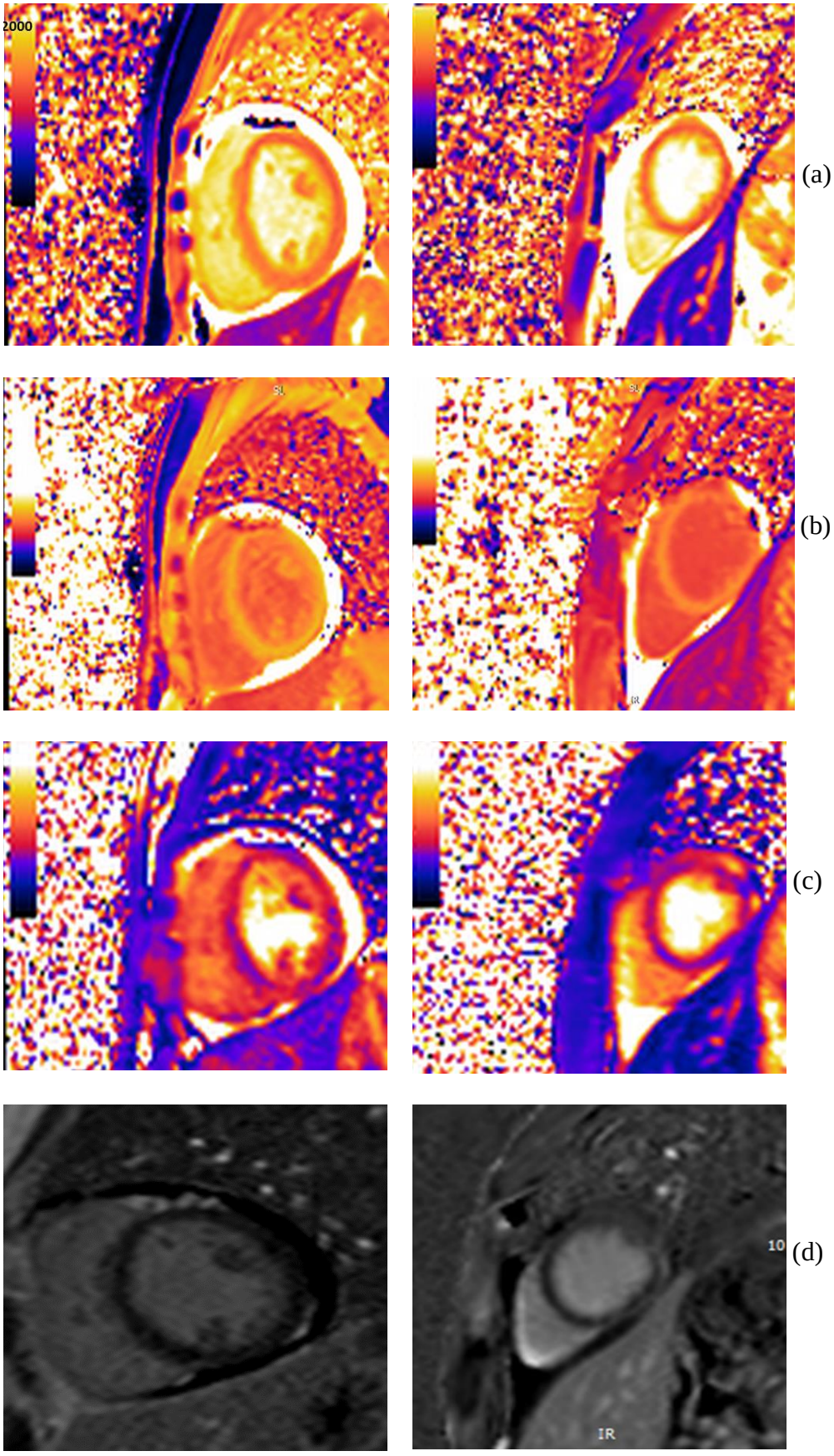


Figure 6.1 Two patients with HIV without treatment (left) with treatment (right), (a) Native T1 images, (b) Postcontrast T1, (c) Native T2, (d) LGE

Fig. 6.1 shows examples of myocardial T1 mapping in a patient with HIV (32 year old female, newly diagnosed HIV infected and not on ART on the left panel) and a patient with HIV (28 year old male with 5 years infected with HIV, on ART for years on the right panel). On the first row, native T1 maps measured 1476±145 ms in the patient not on ART, and 1360±114 ms in the treated patient. On the second row, postcontrast T1 times were measured 749±34 ms in the untreated patient and 647±50 ms in the treated patient. On the third row, T2 relaxation times were measured 45±9 ms and 43±5 ms, respectively. Corresponding LGE images are shown in the fourth row without enhancement in both images.

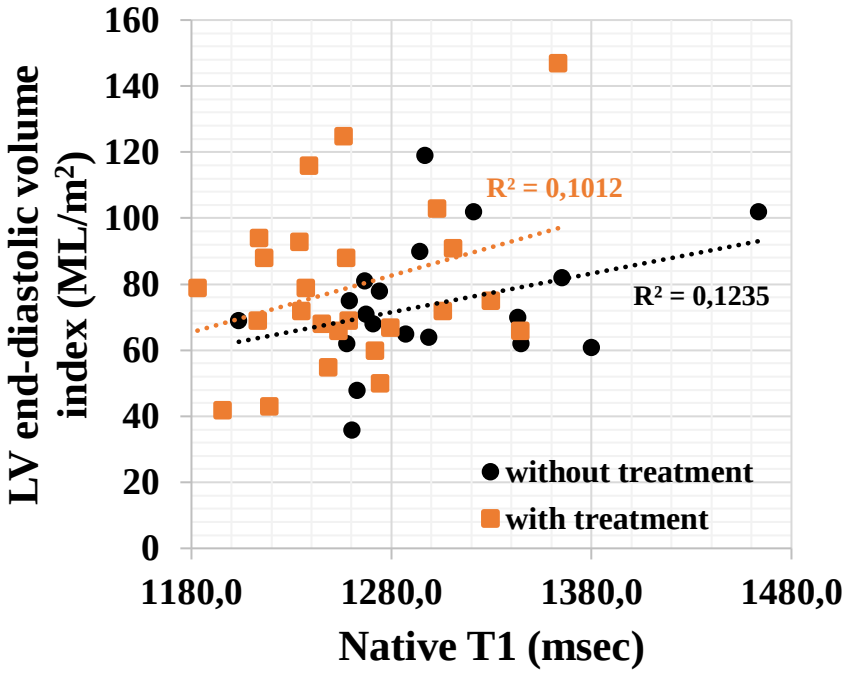


Figure 6.2 Graphs illustrate no correlation between native T1 values and indexed end-diastolic LV volume in the HIV positive patients without and with ART treatment subjects.

As shown in Figures 6.2 and 6.3 there was a no clear significant relation between native T1 values and indexed end-diastolic ([R^2 = 0.12, p-value > 0.1 (with ATR)] and [R^2 = 0.1, p-value > 0.1 (without ART)]), as well as between native T1 and indexed end-systolic volumes ([R^2 = 0.0828, p-value > 0.1 (with ART)] and [R^2 = 0.219, p-value > 0.1 (without ART)]). Also, there was no correlation between native T1 values and LV mass index as shown in Figures 6.4 for

both HIV infected persons without (R^2 = 0.0002 and p-value>0.1) and with (R^2 = 0.016 and p-value>0.1) ART, respectively.

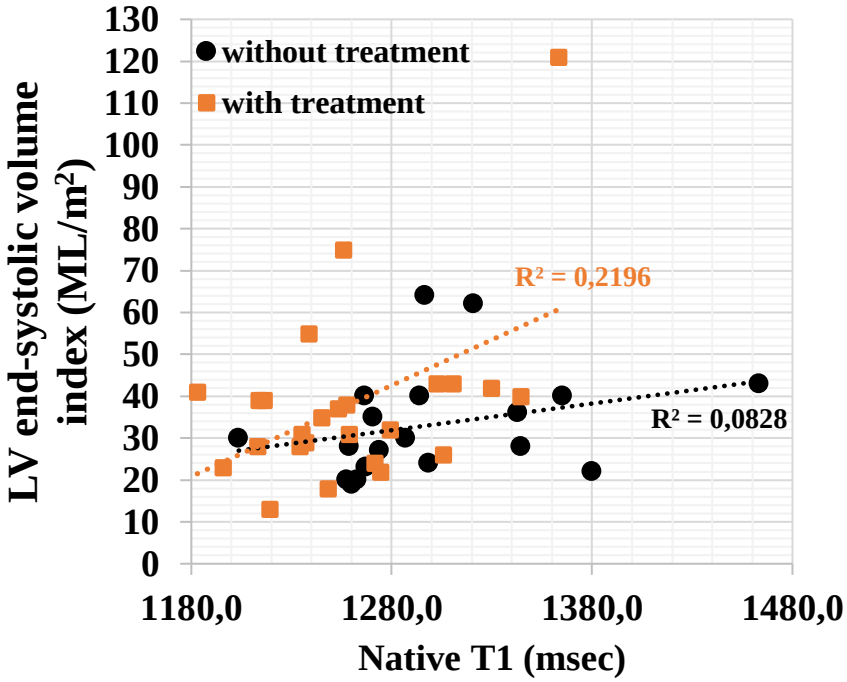


Figure 6.3 Graphs illustrate no correlation between native T1 values and indexed end-systolic LV volume in the HIV positive patients without and with ART treatment subjects.

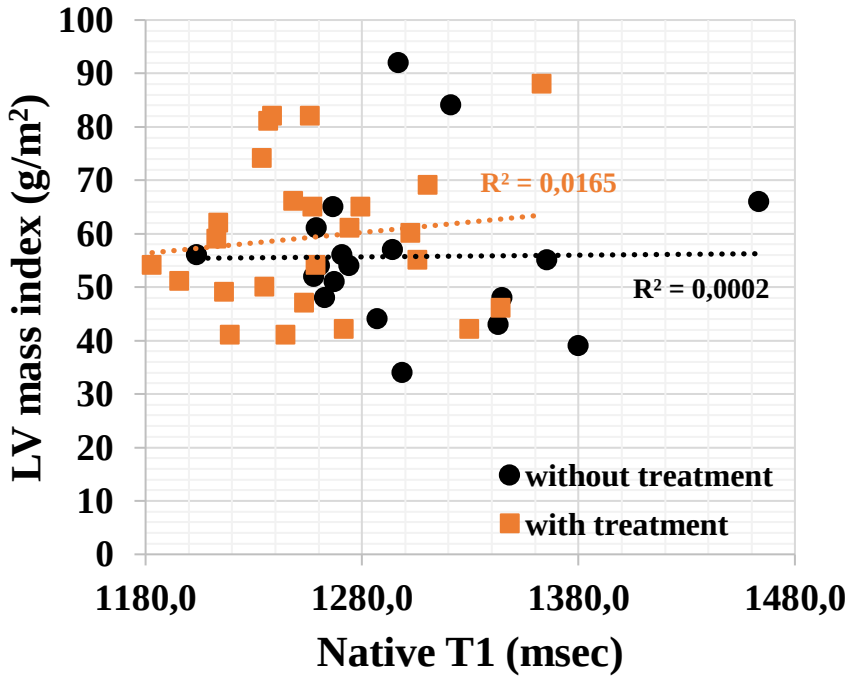


Figure 6.4 Graphs illustrate no correlation between native T1 values and LV mass index without ART and with ART.

The lack of correlation between the fibrosis volume and LV ejection fraction is plotted in Figures 6.5 for the patients without (R^2=0.17 and p-value>0.07) and with (R^2= 0.1155 and p-value>0.1) ART treatment, respectively. For HIV infected patients without ART treatment, the LV ejection fraction decreases with increasing fibrosis volume (Figure 6.5) but for ART treatment has stabilised the effect of the cardiac fibrosis on LV ejection fraction.

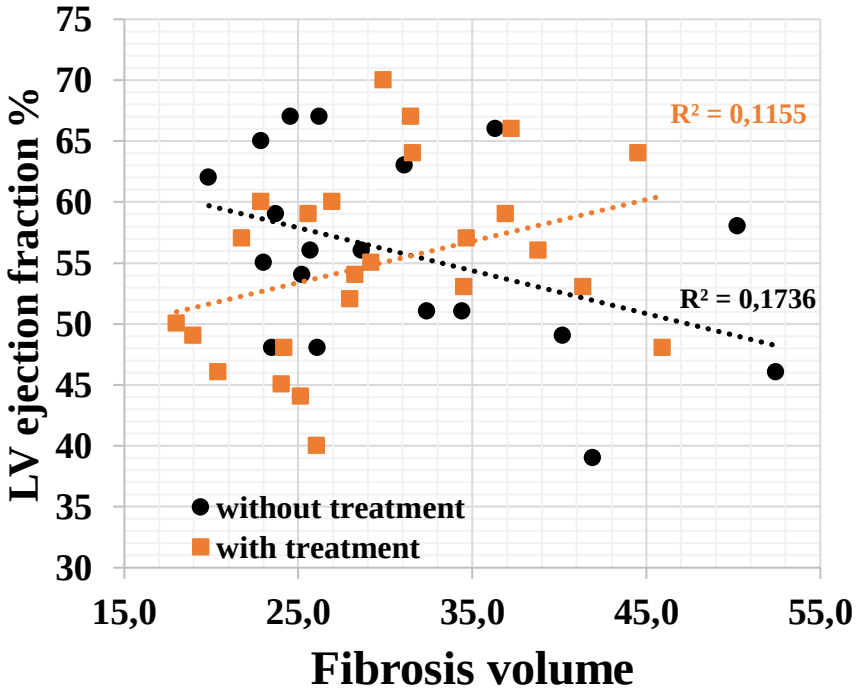


Figure 6.5 Graphs illustrate no correlation between LV ejection fraction with fibrosis volume without ART and with ART.

The native T1 is plotted versus LV ejection fraction in Figure 6.6. There was no significant relationship between native T1 and LV ejection fraction.

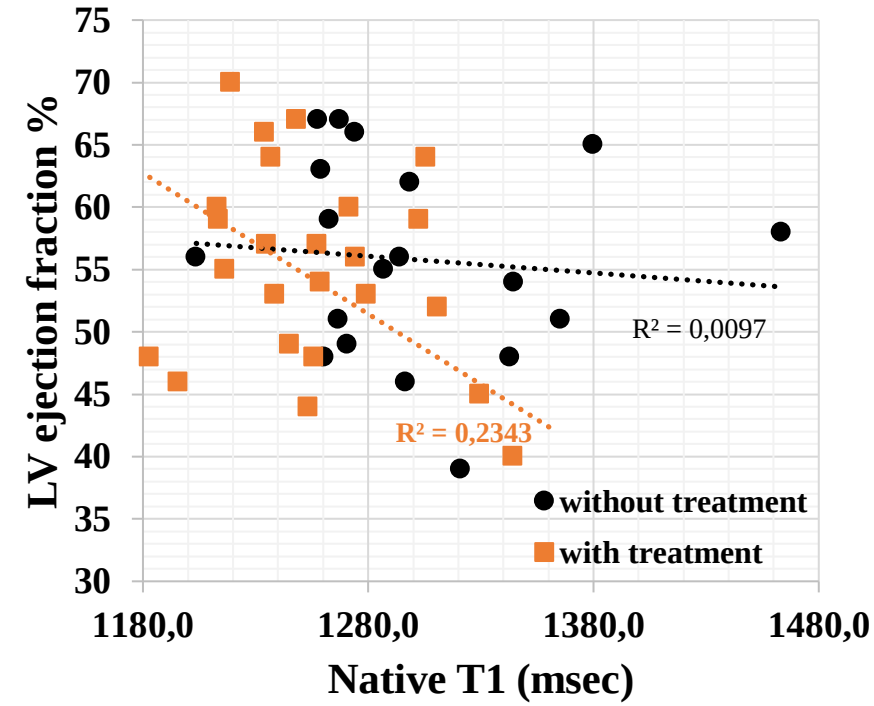


Figure 6.6 Graphs illustrate the correlation between native T1 values and LV mass index without ART with ART.

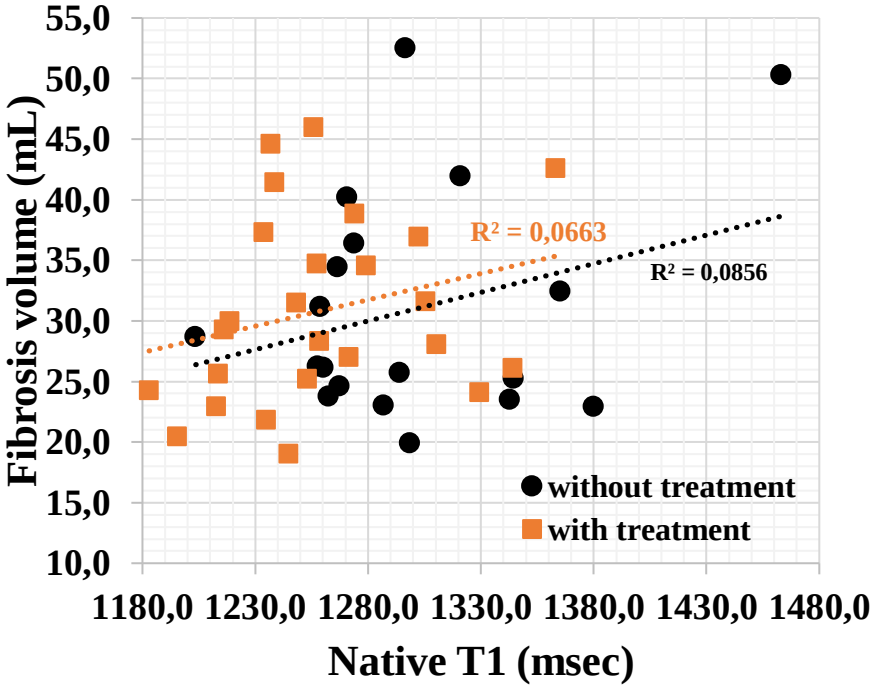


Figure 6.7 Graphs illustrate the correlation between native T1 values and fibrosis volume without ART with ART.

Figure 6.7 shows the plot of the fibrosis volume versus native T1 where the native T1 trend is increasing with the volume of fibrosis, but this does not reach statistical significance. If we compare the Figs. 6.7, we conclude that there is no significant effect of ART on the fibrosis volume (p-value>0.1) where the mean values of fibrosis volume are 31 mL and 30 mL for naive and ART case, respectively.

For more investigation of the effect of ART on diffuse myocardial fibrosis, we plotted the correlation between ECV and CD4 nadir as shown in Figures 6.8. From those correlations, we can confirm that the ART has no significant effect on myocardial fibrosis for the HIV infected patients. While there was a trend for decreasing ECV with increasing of nadir CD4 count, this did not reach statistical significance – see Figures 6.8.

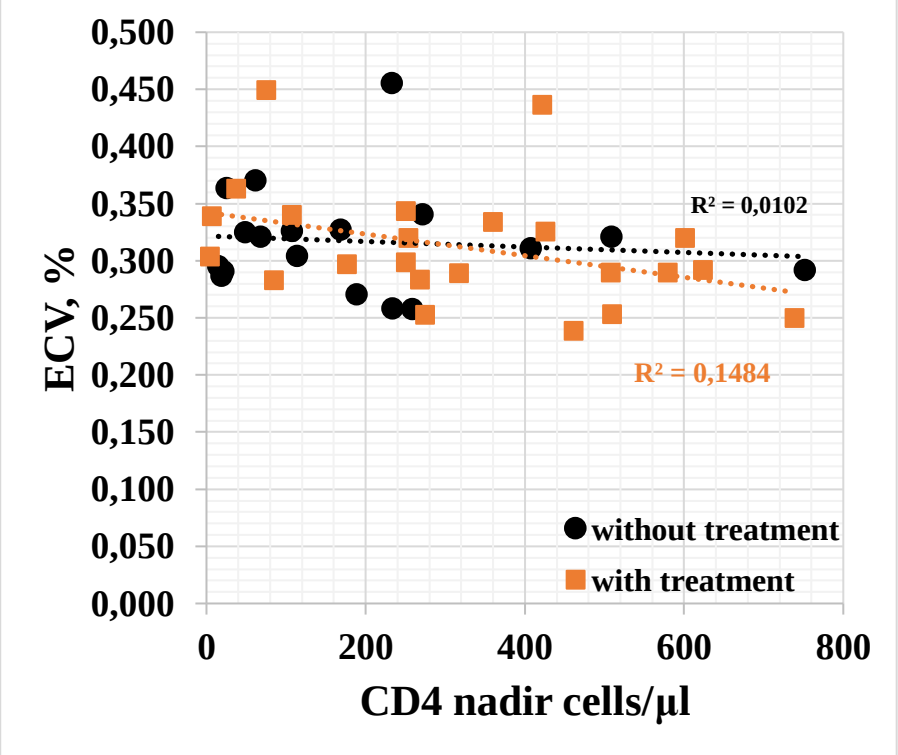


Figure 6.8 Correlation between ECV and CD4 nadir without ART and with ART

Chapter 6: Discussion

In this work, we assessed the impact of ART on diffuse myocardial fibrosis by using CMR. The novel key finding of this study is that native T1 and ECV were elevated in HIV infected patients on ART and in those not yet commenced on ART. The use of ART for over a year was associated with a mild regression in diffuse myocardial fibrosis, as assessed by native T1 values that is lower in patient on ART. Yet, it is still difficult for us to confirm the reason of why T1 values is less for the patients on ART. Native T1 mapping has been utilised for the study of different cardiac conditions that lead to diffuse myocardial fibrosis, including aortic valve stenosis (190), arterial hypertension (191), regurgitant lesions (192), hypertrophic cardiomyopathy and dilated cardiomyopathy (179) . Even though myocardial native T1 values reflect the total composite signal of both intracellular and extracellular compartments, most studies found a significant increase of myocardial T1 values in cardiac diseases with diffuse myocardial fibrosis as compared with T1 values in healthy control subjects. Similarly, increased native T1 values have been reported in patients with asymptomatic aortic valve stenosis (193). Myocardial fibrosis, which has been shown to be a consequence of several chronic inflammatory conditions as well as traditional cardiovascular risk factors (194), is recognised by an accumulation of collagen and has been identified as a contributor to diastolic dysfunction, heart failure, and sudden cardiac death (195).

Native T1 and ECV are the two indices that have been the main focus of clinical research interest in order to obtain better myocardial tissue characterisation. The native, post contrast myocardial/blood T1 values and haematocrit level is used to obtain the ECV based on an important assumption that the contrast concentration reaches equilibrium between blood and tissue. It has been well validated against histological measures of interstitial fibrosis and has

been shown to be a sensitive and robust measurement of collagen volume fraction, whereas post contrast T1 is more susceptible to contrast kinetics (196).

The wide accessibility of ART has decreased AIDS-related complications and deaths. In the pre-ART era, histopathologic findings of myocarditis were reported in >50% of necropsy studies in patients who died from AIDS (197). Studies examining cardiac fibrosis due to myocardial inflammation in patients receiving ART are rare. In one study (42), HIV infected persons were associated with a significantly increased incidence of diffuse rather than focal myocardial fibrosis. In addition, both systolic and diastolic strain and strain rates were impaired (198). However, the relationship between fibrosis in treated and untreated HIV infected patients has not been studied before.

In this cross-sectional study, we evaluated whether ART is related to cardiac fibrosis in HIV infected patients. It still not fully understood if there is correlation between ART and diffuse myocardial fibrosis. From global analysis, we found that diffuse myocardial fibrosis was not different between patient group with ART and without ART.

The novel key finding of this study is that native T1 and ECV were elevated in HIV infected patients on ART and in those not yet commenced not on ART. The use of ART for over a year was associated with a mild regression in diffuse myocardial fibrosis, as assessed by native T1 values that is lower in patient on ART. Yet, it is still difficult for us to confirm the reason of why T1 values is less for the patients on ART.

However, there were no differences between the two groups in the ECV, though the trend was also for lower ECV values in the treated patients. Both ECV and native T1 had no significant relationship with disease duration nor nadir CD4 count. We also assessed the relationship

between myocardial fibrosis and LV remodelling, but could not demonstrate a significant association, perhaps due to the cross-sectional nature of the study. To the best of our knowledge, this is the first and largest study on the extent and impact of ART on diffuse myocardial fibrosis in HIV using CMR. This study has implications for clinical practice and showed that ART may stabilise myocardial fibrosis and that CMR may be used to noninvasively track burden of focal and diffuse myocardial fibrosis in HIV. Our study confirms the previous report by Ntusi's group which obtained a little bit negative correlation between LVEF and Cd4 count nadir (R=-0.23, p=0.03). Furthermore, no associations were obtained between imaging findings and: HIV medication, HIV RNA levels, CD4 nadir or CD4 count (38).

Our findings suggest that CMR without the utilisation of gadolinium-based contrast material may enable precise assessment of diffuse myocardial fibrosis, using native T1 mapping, and that this strategy can be utilised to anticipate the level of subclinical ventricular remodelling and response to therapy. The prognostic effect of estimating native T1 and its relationship with cardiac function in patients associated with having myocardial fibrosis needs more evaluation in future research.

The regression of diffuse myocardial fibrosis assessed with native T1 values was weakly correlated with the ART treatment in HIV positive patients. In general, cardiac fibrosis can be observed more frequently in patients with HIV infection, however, the total volume of fibrosis is relatively low compared to fibrosis in cases of ischemic heart diseases. In our study, there was a nonsignificant difference of ECV values between both patient groups, an indirect measure of diffuse interstitial myocardial fibrosis.

The mechanisms of HIV-associated myocardial dysfunction and fibrosis remain unclear, but the pathogenesis is likely multifactorial: first, subclinical coronary atherosclerosis (199), as well as an increased arterial stiffness (200), have been frequently reported in HIV-infected patients. The findings from this study can provide additional information to the field: ART may influence improving HIV-associated CVD by altering myocardial tissue properties, which include myocardial fibrosis.

Because of the observational and explorative study design, our study has several limitations. This study was performed by using clinical validation for patients diagnosed with HIV. We planned to scan 25 patients without ART, but the national treatment program, for good reasons, has made it difficult to recruit this cohort in our setting. Hence, this study had small numbers. Despite these limitations, these data advance the field and provide justification for using ART, which may stabilise diffuse myocardial fibrosis in HIV. Another major limitation is the fact that the patients on ART were not followed up to establish a causal link between ART use and regression of diffuse myocardial fibrosis. It is possible that the reduction in native T1, may not only indicate reduced diffuse fibrosis, but also decreased subclinical myocardial inflammation.

Another study will be needed with a much longer follow-up (3 years) to show a more overt difference in diffuse cardiac fibrosis with ART. In brief, our finding suggest that ART doesn't have any effect on diffuse myocardial fibrosis within period not less than 1 year on treatment. In future, a larger population at a longer follow-up will be interesting to study the temporal evolution of diffuse myocardial fibrosis. Also, advances in image post-processing have opened new perspective for cardiac MRI.

Chapter 7: Conclusions

The fundamental role in the pathogenesis of heart failure is myocardial fibrosis. Identification and quantification are fundamental for understanding mechanisms of symptoms, prognostication and to improve risk stratification. Previously, this has only been possible with histopathological analysis, with serum biomarkers and echocardiographic parameters unable to provide the diagnostic accuracy required for clinical and scientific application.

Cardiac magnetic resonance imaging provides detailed myocardial tissue characterisation, revolutionising non--invasive analysis of cardiac disease. This methodology has turned into the accepted gold standard for structural and functional cardiac assessment, and we now can visualise the myocardial tissue in an unprecedented detail. Late gadolinium enhancement is established, robust tool to visualise myocardial infarction and predicts recovery of function following revascularisation. Its presence is assumed to identify myocardial scar in non-ischaemic cardiac diseases, with distribution often a key indicator of aetiology.

8.1 Original contribution

To the best of our knowledge, this is the first study to assess the impact of ART in HIV infected patients on diffuse myocardial fibrosis using CMR. We show, for the first time, that ART may result of a regression of diffuse myocardial fibrosis and/or myocardial inflammation.

The results chapter in this thesis have demonstrated that these primary aims have been achieved.

The results have shown that quantitative myocardial tissue assessment using T1 mapping is an independent predictor of cardiac fibrosis with ratio comparable to scar or grey zone quantification detected by conventional LGE-CMR in non-ischemic cardiomyopathy.

Also, Chapter 6 revealed some of the important limitations of T1 mapping in term of imaging. It demonstrated that the pre-contrast and post-contrast T1 maps derived from MOLLI and more

specifically the R1(1/T1) and rΔR1 (R1,pre-R1,post/R1,pre) values could be used for characterisation of different myocardial tissue sub-type in patients with ischaemic cardiomyopathy.

Both LGE and postcontrast T1 mapping recognise myocardial fibrosis. Although, it is likely that diffuse interstitial fibrosis and regional replacement fibrosis will co-exist in many patients with advanced cardiomyopathy, the physiological consequences might be expected to differ.

As expected with all new scientific developments, there is a transformative procedure to optimise methodology and clinical application. This field keeps on being an extremely dynamic research region, with the worldwide point to enhance our diagnostic ability and reduce morbidity and mortality in those with cardiomyopathy. Hopefully, the work exhibited in this thesis will contribute to this process, with ongoing research by the students, supervisors, their extended research group and other independent researchers all contributing to advance our understanding of myocardial fibrosis in heart failure.

8.2 Clinical perspective, implications and limitations

The findings from the thesis provides a potential information to the field of HIV-associated cardiovascular disease by applying CMR imaging parameters to describe subtle differences in myocardial tissue, which include myocardial fibrosis. We suggest that chronic inflammation in the setting of treated HIV likely underlies the findings, which include structural and functional differences and high levels of fibrosis in the setting of treated HIV.

Our study likewise confirms the previous reports of high rates of myocardial fibrosis in HIV. Comparable findings have been reported in other chronic inflammatory conditions such as rheumatoid arthritis.

Regional myocardial fibrosis, conventionally detected by LGE-CMR provides potential substrate for the initiation and maintenance of ventricular arrhythmia. Myocardial tissue

characterization using T1 mapping has emerged as a new CMR application, which has the potential to overcome the limitations of conventional LGE-CMR techniques and characterize diffuse fibrosis.

8.3 Future direction

Like with any rising method, we can just investigate the potentials of T1 mapping through a better understanding of its limitations. However, differences in T1 mapping acquisition sequences and protocols make the absolute T1 values difficult to compare across studies, the evidence presented so far shows great promise for T1 mapping in becoming another cornerstone in the clinical application of CMR, but it needs to be performed in rigorous conditions with accurate post-processing registration in order to improve its efficacy.

Of course, with all new logical improvements, there is a developmental procedure to enhance approach and clinical application. This field keeps on being an exceptionally dynamic research zone, and more studies are needed to build up the" ideal" thresholding the values of diffuse and regional fibrosis.

More studies are needed to build up the "ideal" thresholding values of diffuse and regional fibrosis to enable the translation of noninvasive myocardial fibrosis quantification using LGE-CMR and T1 mapping to the clinical management of patients at risk of life-threatening arrhythmias or predicting CRT positive response.

It is hoped that the work presented in this thesis will contribute to this process, with ongoing research by the candidate, supervisors, their extended research group and other independent researchers all contributing to advance our understanding of the effect of ART on myocardial fibrosis in heart failure for HIV positive patients.

References

1. Gottlieb M, Schanker H, Fan P, Saxon A. Pneumocystis pneumonia--Los Angeles. MMWR Morb [Internet]. 1981 [cited 2017 Aug 11]; Available from: http://www.popline.org/node/421156
2. Cock K De, Jaffe H, Curran J. The evolving epidemiology of HIV/AIDS. Aids [Internet]. 2012 [cited 2017 Aug 11]; Available from: http://journals.lww.com/aidsonline/Abstract/2012/06190/The_evolving_epidemiology_of_HIV_AIDS.9.aspx
3. Johnson L. An introduction to the epidemiology of HIV/AIDS. Cent Actuar [Internet]. 2003 [cited 2017 Aug 11]; Available from: https://www.commerce.uct.ac.za/Research_Units/CARE/RESEARCH/PAPERS/EpidemiologyIntro.doc
4. Ending AIDS: progress towards the 90–90–90 targets | UNAIDS [Internet]. [cited 2018 Feb 12]. Available from: http://www.unaids.org/en/resources/documents/2017/20170720_Global_AIDS_update_2017
5. South Africa | UNAIDS [Internet]. [cited 2017 Aug 14]. Available from: http://www.unaids.org/en/regionscountries/countries/southafrica
6. Poundstone KE, Strathdee SA, Celentano DD. The Social Epidemiology of Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome. Epidemiol Rev [Internet]. 2004 Jul 1 [cited 2017 Jul 31];26(1):22–35. Available from: https://academic.oup.com/epirev/article-lookup/doi/10.1093/epirev/mxh005
7. Neluheni T, Macheka T, Parker W, Abdullah F, Pule M, Motsieloa L. South Africa Global AIDS Response Progress Report (GARPR) 2015 Acknowledgements IHPS Team Members SANAC Team Members. South AfricA Glob AiDS reSponSe Prog Rep [Internet]. 2015 [cited 2017 Sep 28]; Available from: http://sanac.org.za/wp-content/uploads/2016/06/GARPR_report-high-res-for-print-June-15-2016.pdf
8. Seyler C, Anglaret X, Dakoury-Dogbo N, Messou E, Touré S, Danel C, et al. Medium-term survival, morbidity and immunovirological evolution in HIV-infected adults receiving antiretroviral therapy, Abidjan, Côte d'Ivoire. Antivir Ther [Internet]. 2003 Oct [cited 2017 Sep 28];8(5):385–93. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14640385
9. THE GAP REPORT. [cited 2017 Sep 28]; Available from: http://www.unaids.org/sites/default/files/media_asset/UNAIDS_Gap_report_en.pdf
10. Global A. response progress report 2012. Repub South Africa. 2012;
11. Ntsekhe M, Mayosi BM. Cardiac manifestations of HIV infection: an African perspective. Nat Clin Pract Cardiovasc Med [Internet]. 2009 Feb 23 [cited 2017 Aug 1];6(2):120–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19104517
12. Thum T. Noncoding RNAs and myocardial fibrosis. Nat Rev Cardiol [Internet]. 2014 [cited 2017 Jul 31]; Available from: https://www.nature.com/articles/nrcardio.2014.125
13. Ledergerber B, Furrer H, Rickenbach M. Factors associated with the incidence of type 2 diabetes mellitus in HIV-infected participants in the Swiss HIV Cohort Study. Clin Infect [Internet]. 2007 [cited 2017 Jul 31]; Available from: https://academic.oup.com/cid/article-abstract/45/1/111/479264
14. Shalit P. Management of dyslipidemia in patients with human immunodeficiency virus. Rev Cardiovasc Med [Internet]. 2014 [cited 2017 Jul 31];15 Suppl 1:S38-46. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24987862
15. Bloomfield G, Khazanie P, Morris A. HIV and non-communicable cardiovascular and

pulmonary diseases in low-and middle-income countries in the ART era: what we know and best directions. J Acquir [Internet]. 2014 [cited 2017 Jul 31]; Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4133739/
16. Rerkpattanapipat P, Wongpraparut N, Jacobs LE, Kotler MN. Cardiac Manifestations of Acquired Immunodeficiency Syndrome. Arch Intern Med [Internet]. 2000 Mar 13 [cited 2017 Sep 28];160(5). Available from: https://www.avert.org/node/404/pdf
17. Triant VA, Lee H, Hadigan C, Grinspoon SK. Increased Acute Myocardial Infarction Rates and Cardiovascular Risk Factors among Patients with Human Immunodeficiency Virus Disease. J Clin Endocrinol Metab [Internet]. 2007 Jul 1 [cited 2017 Jul 31];92(7):2506–12. Available from: https://academic.oup.com/jcem/article-lookup/doi/10.1210/jc.2006-2190
18. Lewis W. Cardiomyopathy in AIDS: a pathophysiological perspective. Prog Cardiovasc Dis [Internet]. 2000 [cited 2017 Jul 31]; Available from: http://www.sciencedirect.com/science/article/pii/S0033062000000098
19. Hakim JG, Matenga JA, Siziya S. Myocardial dysfunction in human immunodeficiency virus infection: an echocardiographic study of 157 patients in hospital in Zimbabwe. Heart [Internet]. 1996 Aug [cited 2017 Jul 31];76(2):161–5. Available from: http://heart.bmj.com/content/76/2/161.short
20. Magula N, Mayosi B. Cardiac involvement in HIV-infected people living in Africa: a review. Cardiovasc J South Africa [Internet]. 2003 [cited 2017 Jul 31]; Available from: https://journals.co.za/content/cardio/14/5/EJC23868
21. Friis-Møller N, Thiébaut R, Reiss P. Predicting the risk of cardiovascular disease in HIV-infected patients: the data collection on adverse effects of anti-HIV drugs study. Eur J [Internet]. 2010 [cited 2017 Jul 31]; Available from: http://journals.sagepub.com/doi/abs/10.1097/hjr.0b013e328336a150
22. Sliwa K, Carrington MJ, Becker A, Thienemann F, Ntsekhe M, Stewart S. Contribution of the human immunodeficiency virus/acquired immunodeficiency syndrome epidemic to de novo presentations of heart disease in the Heart of Soweto Study cohort. Eur Heart J [Internet]. 2012 Apr 1 [cited 2017 Aug 1];33(7):866–74. Available from: https://academic.oup.com/eurheartj/article-lookup/doi/10.1093/eurheartj/ehr398
23. Hsue PY, Hunt PW, Ho JE, Farah HH, Schnell A, Hoh R, et al. Impact of HIV Infection on Diastolic Function and Left Ventricular Mass. Circ Hear Fail [Internet]. 2010 Jan 1 [cited 2017 Jul 31];3(1):132–9. Available from: http://circheartfailure.ahajournals.org/content/3/1/132.short
24. Longo-Mbenza B, Seghers L, Vita E, Tonduangu K. Assessment of ventricular diastolic function in AIDS patients from Congo: a Doppler echocardiographic study. Heart [Internet]. 1998 [cited 2017 Jul 31]; Available from: http://heart.bmj.com/content/80/2/184.short
25. Hunt PW, Brenchley J, Sinclair E, McCune JM, Roland M, Page-Shafer K, et al. Relationship between T Cell Activation and CD4 + T Cell Count in HIV-Seropositive Individuals with Undetectable Plasma HIV RNA Levels in the Absence of Therapy. J Infect Dis [Internet]. 2008 Jan [cited 2017 Jul 31];197(1):126–33. Available from: https://academic.oup.com/jid/article-abstract/197/1/126/796374
26. López B, González A, Ravassa S, Beaumont J, Moreno MU, San José G, et al. Circulating Biomarkers of Myocardial Fibrosis: The Need for a Reappraisal. J Am Coll Cardiol [Internet]. 2015 Jun 9 [cited 2018 Jun 20];65(22):2449–56. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26046739
27. Mewton N, Liu CY, Croisille P, Bluemke D, Lima JAC. Assessment of myocardial fibrosis with cardiovascular magnetic resonance. J Am Coll Cardiol [Internet]. 2011 Feb 22 [cited 2017 Jul 31];57(8):891–903. Available from:

http://www.sciencedirect.com/science/article/pii/S0735109710048060
28. Spodick DH. Macrophysiology, microphysiology, and anatomy of the pericardium: a synopsis. Am Heart J [Internet]. 1992 Oct 1 [cited 2018 Jun 25];124(4):1046–51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1529878
29. Histology of the Heart Wall | histologyolm.stevegallik.org [Internet]. [cited 2018 Jun 25]. Available from: http://histologyolm.stevegallik.org/node/347
30. Stevens A (Pathologist), Lowe JS (James S, Stevens A (Pathologist). Human histology [Internet]. 2nd ed. London ;;Baltimore: Mosby; 1997 [cited 2018 Jun 25]. 408 p. Available from: http://uct.worldcat.org/title/human-histology/oclc/35652355
31. Bashey RI, Martinez-Hernandez A, Jimenez SA. Isolation, characterization, and localization of cardiac collagen type VI. Associations with other extracellular matrix components. Circ Res [Internet]. 1992 May 1 [cited 2018 Jun 25];70(5):1006–17. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1568294
32. Cohen IS, Anderson DW, Virmani R, Reen BM, Macher AM, Sennesh J, et al. Congestive cardiomyopathy in association with the acquired immunodeficiency syndrome. N Engl J Med [Internet]. 1986 Sep 4 [cited 2017 Aug 1];315(10):628–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3736602
33. Currie PFPF, Jacob AJ, Foreman ARAR, Elton RARA, Brettle RPRP, Boon NA. Heart muscle disease related to HIV infection: prognostic implications. BMJ [Internet]. 1994 Dec 17 [cited 2017 Jul 31];309(6969):1605–7. Available from: http://www.bmj.com/content/309/6969/1605?linkType=FULL&ck=nck&resid=309/6969/1605&journalCode=bmj
34. Reinsch N, Neuhaus K, Esser S, Potthoff A, Hower M, Brockmeyer NH, et al. Prevalence of cardiac diastolic dysfunction in HIV-infected patients: results of the HIV-HEART study. HIV Clin [Internet]. 2010 Jun 6 [cited 2017 Jul 31];11(3):156–62. Available from: http://www.tandfonline.com/doi/abs/10.1310/hct1103-156
35. Remick J, Georgiopoulou V, Marti C, Ofotokun I, Kalogeropoulos A, Lewis W, et al. Heart failure in patients with human immunodeficiency virus infection: epidemiology, pathophysiology, treatment, and future research. Circulation [Internet]. 2014 Apr 29 [cited 2017 Jul 31];129(17):1781–9. Available from: http://circ.ahajournals.org/cgi/doi/10.1161/CIRCULATIONAHA.113.004574
36. Isasti G, Pérez I, Moreno T, Cabrera F. Echocardiographic abnormalities and associated factors in a cohort of asymptomatic HIV-infected patients. AIDS Res [Internet]. 2013 [cited 2017 Jul 31]; Available from: http://online.liebertpub.com/doi/abs/10.1089/aid.2012.0096
37. Bloomfield G, Alenezi F, Barasa F, Lumsden R. Human immunodeficiency virus and heart failure in low-and middle-income countries. JACC Hear Fail [Internet]. 2015 [cited 2017 Jul 31]; Available from: http://www.sciencedirect.com/science/article/pii/S2213177915003236
38. Holloway CJ, Ntusi N, Suttie J, Mahmod M, Wainwright E, Clutton G, et al. Comprehensive cardiac magnetic resonance imaging and spectroscopy reveal a high burden of myocardial disease in HIV patients. Circulation [Internet]. 2013 Aug 20 [cited 2017 Jul 31];128(8):814–22. Available from: http://circ.ahajournals.org/content/circulationaha/early/2013/07/01/CIRCULATIONAHA.113.001719.full.pdf
39. Heart failure in patients with human immunodeficiency virus infection: Epidemiology and management disparities. Int J Cardiol [Internet]. 2016 Sep 1 [cited 2017 Sep 26];218:43–6. Available from: http://www.sciencedirect.com/science/article/pii/S0167527316309299?via%3Dihub
40. Grody WW, Cheng L, Lewis W. Infection of the heart by the human immunodeficiency

virus. Am J Cardiol [Internet]. 1990 Jul 15 [cited 2018 Feb 16];66(2):203–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2371952
41. Grinspoon S, Mulligan K. Weight Loss and Wasting in Patients Infected with Human Immunodeficiency Virus. Clin Infect Dis [Internet]. 2003 Apr 1 [cited 2017 Nov 14];36(s2):S69–78. Available from: https://academic.oup.com/cid/article-lookup/doi/10.1086/367561
42. Thiara DK, Liu CY, Raman F, Mangat S, Purdy JB, Duarte HA, et al. Abnormal Myocardial Function Is Related to Myocardial Steatosis and Diffuse Myocardial Fibrosis in HIV-Infected Adults. J Infect Dis [Internet]. 2015 Nov 15 [cited 2018 Feb 1];212(10):1544–51. Available from: https://academic.oup.com/jid/article-lookup/doi/10.1093/infdis/jiv274
43. Lipshultz SE, Mas CM, Henkel JM, Franco VI, Fisher SD, Miller TL. HAART to heart: highly active antiretroviral therapy and the risk of cardiovascular disease in HIV-infected or exposed children and adults. Expert Rev Anti Infect Ther [Internet]. 2012 Jun 10 [cited 2018 Jan 26];10(6):661–74. Available from: http://www.tandfonline.com/doi/full/10.1586/eri.12.53
44. Ntusi NAB. HIV and myocarditis. Curr Opin HIV AIDS [Internet]. 2017 [cited 2017 Sep 21];12(6). Available from: http://insights.ovid.com/crossref?an=01222929-201711000-00010
45. Butt AAA, Chang C-CC, Kuller L, Goetz MBM, Leaf D, Rimland D, et al. Risk of Heart Failure With Human Immunodeficiency Virus in the Absence of Prior Diagnosis of Coronary Heart Disease. Arch Intern Med [Internet]. 2011 Apr 25 [cited 2017 Aug 1];171(8). Available from: http://archinte.jamanetwork.com/article.aspx?doi=10.1001/archinternmed.2011.151
46. Mondy KE, Gottdiener J, Overton ET, Henry K, Bush T, Conley L, et al. High Prevalence of Echocardiographic Abnormalities among HIV-infected Persons in the Era of Highly Active Antiretroviral Therapy. Clin Infect Dis [Internet]. 2011 Feb 1 [cited 2017 Jul 31];52(3):378–86. Available from: https://academic.oup.com/cid/article-abstract/52/3/378/304946
47. Barbaro G. HIV-associated myocarditis. Heart Fail Clin [Internet]. 2005 Oct [cited 2017 Aug 1];1(3):439–48. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1551713605000383
48. BARBARO G, DI LORENZO G, GRISORIO B, BARBARINI G. Cardiac Involvement in the Acquired Immunodeficiency Syndrome: A Multicenter Clinical-Pathological Study. AIDS Res Hum Retroviruses [Internet]. 1998 Aug 10 [cited 2017 Jul 31];14(12):1071–7. Available from: http://online.liebertpub.com/doi/abs/10.1089/aid.1998.14.1071
49. Hofman P, Drici MD, Gibelin P, Michiels JF, Thyss A. Prevalence of toxoplasma myocarditis in patients with the acquired immunodeficiency syndrome. Heart [Internet]. 1993 Oct 1 [cited 2018 Jan 16];70(4):376–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8217449
50. Kinney EL, Monsuez JJ, Kitzis M, Vittecoq D. Treatment of AIDS-associated heart disease. Angiology [Internet]. 1989 Nov 2 [cited 2018 Jan 16];40(11):970–6. Available from: http://journals.sagepub.com/doi/10.1177/000331978904001106
51. Freedberg RS, Gindea AJ, Dieterich DT, Greene JB. Herpes simplex pericarditis in AIDS. N Y State J Med [Internet]. 1987 May [cited 2018 Jan 16];87(5):304–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3035442
52. Barbaro G, Di Lorenzo G, Soldini M, Giancaspro G, Grisorio B, Pellicelli A, et al. Intensity of myocardial expression of inducible nitric oxide synthase influences the clinical course of human immunodeficiency virus-associated cardiomyopathy. Gruppo

Italiano per lo Studio Cardiologico dei pazienti affetti da AIDS (GISCA). Circulation [Internet]. 1999 Aug 31 [cited 2018 Jan 16];100(9):933–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10468523
53. Barbaro G. Cardiovascular manifestations of HIV infection. J R Soc Med [Internet]. 2001 Aug [cited 2018 Jan 16];94(8):384–90. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11461981
54. Adair O V, Randive N, Krasnow N. Isolated toxoplasma myocarditis in acquired immune deficiency syndrome. Am Heart J [Internet]. 1989 Oct [cited 2018 Jan 16];118(4):856–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2801493
55. Magnani JW, Dec GW. Myocarditis: current trends in diagnosis and treatment. Circulation [Internet]. 2006 Feb 14 [cited 2017 Jul 31];113(6):876–90. Available from: http://circ.ahajournals.org/cgi/doi/10.1161/CIRCULATIONAHA.105.584532
56. Lipshultz SE, Miller TL, Wilkinson JD, Scott GB, Somarriba G, Cochran TR, et al. Cardiac effects in perinatally HIV-infected and HIV-exposed but uninfected children and adolescents: a view from the United States of America. J Int AIDS Soc [Internet]. 2013 Jun 18 [cited 2017 Aug 7];16(1):18597. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23782480
57. Pugliese A, Isnardi D, Saini A, Scarabelli T, Raddino R. Impact of highly active antiretroviral therapy in HIV-positive patients with cardiac involvement. J Infect [Internet]. 2000 [cited 2017 Jul 31]; Available from: http://www.sciencedirect.com/science/article/pii/S0163445300906728
58. Ntusi N, O'Dwyer E, … LD-C, 2016 undefined. HIV-1–Related Cardiovascular Disease Is Associated With Chronic Inflammation, Frequent Pericardial Effusions, and Probable Myocardial EdemaCLINICAL. Am Hear Assoc [Internet]. [cited 2018 Jan 18]; Available from: http://circimaging.ahajournals.org/user/logout?current=node/252446
59. Leite-Moreira A. Current perspectives in diastolic dysfunction and diastolic heart failure. Heart [Internet]. 2006 [cited 2017 Jul 31]; Available from: http://heart.bmj.com/content/92/5/712.short
60. Redfield M, Jacobsen S, Jr JB. Burden of systolic and diastolic ventricular dysfunction in the community: appreciating the scope of the heart failure epidemic. Jama [Internet]. 2003 [cited 2017 Jul 31]; Available from: http://jamanetwork.com/journals/jama/fullarticle/195749
61. Luo L, Ye Y, Liu Z, Zuo L, Li Y, Han Y, et al. Assessment of cardiac diastolic dysfunction in HIV-infected people without cardiovascular symptoms in China. Int J STD AIDS [Internet]. 2010 Dec [cited 2017 Sep 20];21(12):814–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21297089
62. Blaylock JM, Byers DK, Gibbs BT, Nayak G, Ferguson M, Tribble DR, et al. Longitudinal assessment of cardiac diastolic function in HIV-infected patients. Int J STD AIDS [Internet]. 2012 Feb [cited 2017 Sep 20];23(2):105–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22422684
63. Daneshvar D, Wei J, Tolstrup K, Thomson LEJ, Shufelt C, Merz CNB. Diastolic dysfunction: improved understanding using emerging imaging techniques. Am Heart J [Internet]. 2010 Sep [cited 2017 Jul 31];160(3):394–404. Available from: http://www.sciencedirect.com/science/article/pii/S0002870310005569
64. Nayak G, Ferguson M, Tribble D. Cardiac diastolic dysfunction is prevalent in HIV-infected patients. AIDS Patient Care [Internet]. 2009 [cited 2017 Jul 31]; Available from: http://online.liebertpub.com/doi/abs/10.1089/apc.2008.0142
65. Seaberg E, Munoz A, Lu M, Detels R, Margolick J. Association between highly active antiretroviral therapy and hypertension in a large cohort of men followed from 1984 to 2003. Aids [Internet]. 2005 [cited 2017 Jul 31]; Available from:

http://journals.lww.com/aidsonline/Abstract/2005/06100/Association_between_highly_active_antiretroviral.13.aspx
66. Russo C, Jin Z, Homma S, Rundek T, Elkind MS V, Sacco RL, et al. Effect of obesity and overweight on left ventricular diastolic function: a community-based study in an elderly cohort. J Am Coll Cardiol [Internet]. 2011 Mar 22 [cited 2018 Jun 25];57(12):1368–74. Available from: https://www.sciencedirect.com/science/article/pii/S0735109711000957?via%3Dihub
67. Lorenz M, Stephan C, Harmjanz A, Staszewski S. Both long-term HIV infection and highly active antiretroviral therapy are independent risk factors for early carotid atherosclerosis. Atherosclerosis [Internet]. 2008 [cited 2017 Jul 31]; Available from: http://www.sciencedirect.com/science/article/pii/S0021915007000317
68. Fontes-Carvalho R, Mancio J, Marcos A. HIV patients have impaired diastolic function that is not aggravated by anti-retroviral treatment. drugs Ther [Internet]. 2015 [cited 2017 Jul 31]; Available from: http://link.springer.com/article/10.1007/s10557-015-6573-x
69. Weber KT, Sun Y, Bhattacharya SK, Ahokas RA, Gerling IC. Myofibroblast-mediated mechanisms of pathological remodelling of the heart. Nat Rev Cardiol [Internet]. 2013 Jan 4 [cited 2017 Aug 1];10(1):15–26. Available from: http://www.nature.com/doifinder/10.1038/nrcardio.2012.158
70. Rozario T, DeSimone DW. The extracellular matrix in development and morphogenesis: A dynamic view. Dev Biol [Internet]. 2010 May 1 [cited 2018 Jun 29];341(1):126–40. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19854168
71. Kong P, Christia P, Frangogiannis NG. The pathogenesis of cardiac fibrosis. Cell Mol Life Sci [Internet]. 2014 Feb 7 [cited 2017 Jul 31];71(4):549–74. Available from: http://link.springer.com/article/10.1007/s00018-013-1349-6
72. Rossi MA. Connective tissue skeleton in the normal left ventricle and in hypertensive left ventricular hypertrophy and chronic chagasic myocarditis [Internet]. Vol. 7. 2001 [cited 2017 Oct 4]. p. 820–32. Available from: https://www.scopus.com/record/display.uri?eid=2-s2.0-0034938820&origin=inward&txGid=aa9c65609078ed9f24fa77866694bc13
73. Wynn TA. Cellular and molecular mechanisms of fibrosis. J Pathol [Internet]. 2008 Jan [cited 2018 Jun 25];214(2):199–210. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18161745
74. Mewton N, Liu CYC, Croisille P, Bluemke D, Lima JACC. Assessment of myocardial fibrosis with cardiovascular magnetic resonance. J Am Coll Cardiol [Internet]. 2011 Feb 22 [cited 2017 Jul 31];57(8):891–903. Available from: http://www.sciencedirect.com/science/article/pii/S0735109710048060
75. Raj V, Joshi S, Pennell DJ. Images in Cardiovascular Medicine. Cardiac magnetic resonance of acute myocarditis in an human immunodeficiency virus patient presenting with acute chest pain syndrome. Circulation [Internet]. 2010 Jun 29 [cited 2018 Apr 12];121(25):2777–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20585023
76. Himelman RB, Chung WS, Chernoff DN, Schiller NB, Hollander H. Cardiac manifestations of human immunodeficiency virus infection: a two-dimensional echocardiographic study. J Am Coll Cardiol [Internet]. 1989 Apr [cited 2017 Jul 31];13(5):1030–6. Available from: http://www.sciencedirect.com/science/article/pii/0735109789902568
77. Corallo S, Mutinelli MR, Moroni M, Lazzarin A, Celano V, Repossini A, et al. Echocardiography detects myocardial damage in AIDS: prospective study in 102 patients. Eur Heart J [Internet]. 1988 Aug [cited 2017 Jul 31];9(8):887–92. Available from: https://academic.oup.com/eurheartj/article-abstract/9/8/887/476721

78. Hsia J, Ross AM. Pericardial effusion and pericardiocentesis in human immunodeficiency virus infection. Am J Cardiol [Internet]. 1994 Jul 1 [cited 2017 Jul 31];74(1):94–6. Available from: http://www.sciencedirect.com/science/article/pii/0002914994905010
79. Heidenreich PA, Eisenberg MJ, Kee LL, Somelofski CA, Hollander H, Schiller NB, et al. Pericardial effusion in AIDS. Incidence and survival. Circulation [Internet]. 1995 Dec 1 [cited 2017 Jul 31];92(11):3229–34. Available from: http://circ.ahajournals.org/content/92/11/3229.short
80. Bijl M, Dieleman J, Simoons M. Low prevalence of cardiac abnormalities in an HIV-seropositive population on antiretroviral combination therapy. JAIDS J [Internet]. 2001 [cited 2017 Jul 31]; Available from: http://journals.lww.com/jaids/Citation/2001/07010/Low_Prevalence_of_Cardiac_Abnormalities_in_an.18.aspx
81. Reuter H, Burgess LJ, Louw VJ, Doubell AF. The management of tuberculous pericardial effusion: experience in 233 consecutive patients. Cardiovasc J S Afr [Internet]. [cited 2017 Aug 7];18(1):20–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17392991
82. Corey GR, Campbell PT, Van Trigt P, Kenney RT, O'Connor CM, Sheikh KH, et al. Etiology of large pericardial effusions. Am J Med [Internet]. 1993 Aug [cited 2018 Jun 27];95(2):209–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8356985
83. McLaughlin V V., Archer SL, Badesch DB, Barst RJ, Farber HW, Lindner JR, et al. ACCF/AHA 2009 Expert Consensus Document on Pulmonary Hypertension: A Report of the American College of Cardiology Foundation Task Force on Expert Consensus Documents and the American Heart Association: Developed in Collaboration With the American College . Circulation [Internet]. 2009 Apr 28 [cited 2018 Feb 2];119(16):2250–94. Available from: http://circ.ahajournals.org/cgi/doi/10.1161/CIRCULATIONAHA.109.192230
84. D'amati G, Gioia C, Gallo P. Pathological Findings of HIV-Associated Cardiovascular Disease. New York Acad … [Internet]. 2001 [cited 2017 Jul 31]; Available from: http://onlinelibrary.wiley.com/doi/10.1111/j.1749-6632.2001.tb03901.x/full
85. Kreinin S. Tuberculous Pericarditis: Observed from the Stage of Effusion to Pericardial Calcification. Am Rev Respir Dis [Internet]. 1960 [cited 2017 Jul 31]; Available from: http://www.atsjournals.org/doi/pdf/10.1164/arrd.1960.81.4.585
86. Syed F, Mayosi B. A modern approach to tuberculous pericarditis. Prog Cardiovasc Dis [Internet]. 2007 [cited 2017 Jul 31]; Available from: http://www.sciencedirect.com/science/article/pii/S0033062007000266
87. Ntsekhe M, Hakim J. Impact of human immunodeficiency virus infection on cardiovascular disease in Africa. Circulation [Internet]. 2005 [cited 2017 Jul 31]; Available from: http://circ.ahajournals.org/content/112/23/3602.short
88. Park Y, Park S, Kim H, Lee B. Acute Idiopathic Hemorrhagic Pericarditis with Cardiac Tamponade as the Initial Presentation of Acquired Immune Deficiency Syndrome. Yonsei Med [Internet]. 2010 [cited 2017 Jul 31]; Available from: https://synapse.koreamed.org/DOIx.php?id=10.3349/ymj.2010.51.2.273
89. Jr FP, Delaney K, Moorman A. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. Engl J … [Internet]. 1998 [cited 2017 Jul 31]; Available from: http://www.nejm.org/doi/full/10.1056/NEJM199803263381301
90. Fauci A. The AIDS epidemic—considerations for the 21st century. N Engl J Med [Internet]. 1999 [cited 2017 Jul 31]; Available from: http://www.nejm.org/doi/full/10.1056/nejm199909303411406

91. Cammarosano C, Lewis W. Cardiac lesions in acquired immune deficiency syndrome (AIDS). J Am Coll Cardiol [Internet]. 1985 [cited 2017 Aug 1]; Available from: http://www.sciencedirect.com/science/article/pii/S0735109785803971
92. Zumla A, Malon P, Henderson J. Impact of HIV infection on tuberculosis. Postgrad Med [Internet]. 2000 [cited 2017 Aug 1]; Available from: http://pmj.bmj.com/content/76/895/259.short
93. Wynn T. Fibrotic disease and the TH1/TH2 paradigm. Nat Rev Immunol [Internet]. 2004 [cited 2017 Jul 31]; Available from: https://www.nature.com/articles/nri1412
94. Reuter H, Burgess L, Schneider J, Vuuren W. The role of histopathology in establishing the diagnosis of tuberculous pericardial effusions in the presence of HIV. 2006 [cited 2017 Jul 31]; Available from: http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2559.2005.02320.x/full
95. Ntsekhe M, Wiysonge C, Gumedze F, Maartens G. HIV infection is associated with a lower incidence of constriction in presumed tuberculous pericarditis: a prospective observational study. PLoS [Internet]. 2008 [cited 2017 Jul 31]; Available from: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0002253
96. J.M. M, A. del R, Miro JM, del Rio A, Mestres CA. Infective endocarditis and cardiac surgery in intravenous drug abusers and HIV-1 infected patients. Cardiol Clin [Internet]. 2003 [cited 2017 Jul 31];21(2):167–vi. Available from: https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=+Infective+endocarditis+and+cardiac+surgery+in+intravenous+drug+abusers+and+HIV-1+infected+patients.+&btnG=
97. Fisher SD, Kanda BS, Miller TL, Lipshultz SE. Cardiovascular disease and therapeutic drug-related cardiovascular consequences in HIV-infected patients. Am J Cardiovasc Drugs [Internet]. 2011 Dec 1 [cited 2018 Feb 23];11(6):383–94. Available from: http://link.springer.com/10.2165/11594590-000000000-00000
98. Herskowitz A, Willoughby S, Vlahov D. Dilated heart muscle disease associated with HIV infection. Eur Hear [Internet]. 1995 [cited 2017 Aug 1]; Available from: https://academic.oup.com/eurheartj/article-abstract/16/suppl_O/50/442038
99. Khunnawat C, Mukerji S, Havlichek D, Touma R, Abela GS. Cardiovascular manifestations in human immunodeficiency virus-infected patients. Am J Cardiol [Internet]. 2008 Sep 1 [cited 2018 Jun 27];102(5):635–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18721528
100. Gebo KA, Burkey MD, Lucas GM, Moore RD, Wilson LE. Incidence of, Risk Factors for, Clinical Presentation, and 1-Year Outcomes of Infective Endocarditis in an Urban HIV Cohort. JAIDS J Acquir Immune Defic Syndr [Internet]. 2006 Dec [cited 2017 Jul 31];43(4):426–32. Available from: https://insights.ovid.com/crossref?an=00126334-200612010-00007
101. Sudano I, Spieker L, Noll G, Corti R, Weber R. Cardiovascular disease in HIV infection. Am Hear [Internet]. 2006 [cited 2017 Aug 1]; Available from: http://www.sciencedirect.com/science/article/pii/S0002870305007581
102. Pellicelli A, Palmieri F, Cicalini S. Pathogenesis of HIV-Related Pulmonary Hypertension. 2001 [cited 2017 Jul 31]; Available from: http://onlinelibrary.wiley.com/doi/10.1111/j.1749-6632.2001.tb03904.x/full
103. Le Houssine P, Karmochkine M, Ledru F, Batisse D, Piketty C, Kazatchkine MD, et al. [Primary pulmonary hypertension in human immunodeficiency virus infection. Study of 9 cases amd review of the literature]. La Rev Med interne [Internet]. 2001 Dec [cited 2018 Mar 15];22(12):1196–203. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11794890
104. Barbaro G. Reviewing the clinical aspects of HIV-associated pulmonary hypertension:

will the latest therapies prove beneficial? J Respir Dis [Internet]. 2004 [cited 2017 Jul 31]; Available from: http://go.galegroup.com/ps/i.do?id=GALE%7CA134576099&sid=googleScholar&v=2.1&it=r&linkaccess=fulltext&issn=0194259X&p=AONE&sw=w
105. Klatt E. Cardiovascular pathology in AIDS. HIV Infect Cardiovasc Syst [Internet]. 2003 [cited 2017 Jul 31]; Available from: http://www.karger.com/Article/Abstract/73174
106. Cicalini S, Almodovar S, Grilli E, Flores S. Pulmonary hypertension and human immunodeficiency virus infection: epidemiology, pathogenesis, and clinical approach. Clin Microbiol Infect [Internet]. 2011 Jan 1 [cited 2017 Aug 7];17(1):25–33. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20545962
107. Pellicelli AM, Barbaro G, Palmieri F, Girardi E, D'Ambrosio C, Rianda A, et al. Primary Pulmonary Hypertension in HIV Patients: A Systematic Review. Angiology [Internet]. 2001 Jan 2 [cited 2018 Jun 27];52(1):31–41. Available from: http://journals.sagepub.com/doi/10.1177/000331970105200105
108. Petitpretz P, Brenot F, Azarian R, Parent F, Rain B. Pulmonary hypertension in patients with human immunodeficiency virus infection. Comparison with primary pulmonary hypertension. Circulation [Internet]. 1994 [cited 2017 Jul 31]; Available from: http://circ.ahajournals.org/content/89/6/2722.short
109. Zuber J, Calmy A, Evison JM, Hasse B, Schiffer V, Wagels T, et al. Pulmonary Arterial Hypertension Related to HIV Infection: Improved Hemodynamics and Survival Associated with Antiretroviral Therapy. Clin Infect Dis [Internet]. 2004 Apr 15 [cited 2018 Feb 5];38(8):1178–85. Available from: https://academic.oup.com/cid/article-lookup/doi/10.1086/383037
110. Degano B, Guillaume M, Savale L, Montani D, Jaïs X, Yaici A, et al. HIV-associated pulmonary arterial hypertension: survival and prognostic factors in the modern therapeutic era. AIDS [Internet]. 2010 Jan [cited 2018 Feb 5];24(1):67–75. Available from: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00002030-201001020-00009
111. McLaughlin V V., Archer SL, Badesch DB, Barst RJ, Farber HW, Lindner JR, et al. ACCF/AHA 2009 Expert Consensus Document on Pulmonary Hypertension. A Report of the American College of Cardiology Foundation Task Force on Expert Consensus Documents and the American Heart Association Developed in Collaboration With the American College o [Internet]. Vol. 53, Journal of the American College of Cardiology. Elsevier; 2009 [cited 2018 Feb 12]. p. 1573–619. Available from: https://www.sciencedirect.com/science/article/pii/S0735109709001429?via%3Dihub
112. d'Arminio A, Sabin CA, Phillips AN, Reiss P, Weber R, Kirk O, et al. Cardio- and cerebrovascular events in HIV-infected persons. AIDS [Internet]. 2004 Sep 3 [cited 2018 Jun 27];18(13):1811–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15316342
113. Periard D, Cavassini M, Taffe P, Chevalley M, Senn L, Chapuis-Taillard C, et al. High Prevalence of Peripheral Arterial Disease in HIV-Infected Persons. Clin Infect Dis [Internet]. 2008 Mar 1 [cited 2018 Jun 27];46(5):761–7. Available from: https://academic.oup.com/cid/article-lookup/doi/10.1086/527564
114. DAD Study Group, Friis-Møller N, Reiss P, Sabin CA, Weber R, Monforte A d'Arminio, et al. Class of Antiretroviral Drugs and the Risk of Myocardial Infarction. N Engl J Med [Internet]. 2007 Apr 26 [cited 2018 Jun 26];356(17):1723–35. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17460226
115. Hazenberg MD, Stuart JW, Otto SA, Borleffs JC, Boucher CA, de Boer RJ, et al. T-cell division in human immunodeficiency virus (HIV)-1 infection is mainly due to immune

activation: a longitudinal analysis in patients before and during highly active antiretroviral therapy (HAART). Blood [Internet]. 2000 Jan 1 [cited 2018 Jun 26];95(1):249–55. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10607709
116. Park IW, Wang JF, Groopman JE. HIV-1 Tat promotes monocyte chemoattractant protein-1 secretion followed by transmigration of monocytes. Blood [Internet]. 2001 Jan 15 [cited 2018 Jun 26];97(2):352–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11154208
117. Kaplan RC, Sinclair E, Landay AL, Lurain N, Sharrett AR, Gange SJ, et al. T cell activation predicts carotid artery stiffness among HIV-infected women. Atherosclerosis [Internet]. 2011 Jul [cited 2018 Jun 26];217(1):207–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21492857
118. Hsue PY, Deeks SG, Hunt PW. Immunologic Basis of Cardiovascular Disease in HIV-Infected Adults. J Infect Dis [Internet]. 2012 Jun 1 [cited 2018 Jun 26];205(suppl_3):S375–82. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22577211
119. Kaplan RC, Sinclair E, Landay AL, Lurain N, Sharrett AR, Gange SJ, et al. T cell activation and senescence predict subclinical carotid artery disease in HIV-infected women. J Infect Dis [Internet]. 2011 Feb 15 [cited 2018 Jun 26];203(4):452–63. Available from: https://academic.oup.com/jid/article-lookup/doi/10.1093/infdis/jiq071
120. Kaplan RC, Kingsley LA, Gange SJ, Benning L, Jacobson LP, Lazar J, et al. Low CD4+ T-cell count as a major atherosclerosis risk factor in HIV-infected women and men. AIDS [Internet]. 2008 Aug 20 [cited 2018 Jun 27];22(13):1615–24. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18670221
121. Neuhaus J, Jacobs DR, Baker J V., Calmy A, Duprez D, La Rosa A, et al. Markers of inflammation, coagulation, and renal function are elevated in adults with HIV infection. J Infect Dis [Internet]. 2010 Jun 15 [cited 2018 Jun 20];201(12):1788–95. Available from: https://academic.oup.com/jid/article-lookup/doi/10.1086/652749
122. Calza L, Manfredi R, Verucchi G. Myocardial infarction risk in HIV-infected patients: epidemiology, pathogenesis, and clinical management. AIDS. 2010 Mar;24(6):789–802.
123. Riddler SA, Smit E, Cole SR, Li R, Chmiel JS, Dobs A, et al. Impact of HIV Infection and HAART on Serum Lipids in Men. JAMA [Internet]. 2003 Jun 11 [cited 2018 Jun 27];289(22):2978. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12799406
124. Becker AC, Jacobson B, Singh S, Sliwa K, Stewart S, Libhaber E, et al. The Thrombotic Profile of Treatment-Naive HIV-Positive Black South Africans With Acute Coronary Syndromes. Clin Appl Thromb [Internet]. 2011 Jun 11 [cited 2018 Jun 27];17(3):264–72. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20460356
125. National Antiretroviral Treatment Guidelines FIRST EDITION. [cited 2017 Aug 14]; Available from: http://apps.who.int/medicinedocs/documents/s17758en/s17758en.pdf
126. Barbaro G, Barbarini G. Human immunodeficiency virus & cardiovascular risk. Indian J Med Res [Internet]. 2011 [cited 2017 Jul 31]; Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/pmc3284097/
127. Worm SW, Sabin C, Weber R, Reiss P, El-Sadr W, Dabis F, et al. Risk of Myocardial Infarction in Patients with HIV Infection Exposed to Specific Individual Antiretroviral Drugs from the 3 Major Drug Classes: The Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) Study. J Infect Dis [Internet]. 2010 Feb 1 [cited 2017 Aug 3];201(3):318–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20039804
128. Choi AI, Vittinghoff E, Deeks SG, Weekley CC, Li Y, Shlipak MG. Cardiovascular risks associated with abacavir and tenofovir exposure in HIV-infected persons. AIDS [Internet]. 2011 Jun 19 [cited 2017 Aug 3];25(10):1289–98. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/21516027
129. Carr A, Cooper D. Adverse effects of antiretroviral therapy. Lancet [Internet]. 2000 [cited 2017 Aug 1]; Available from: http://www.sciencedirect.com/science/article/pii/S0140673600028543
130. Constans J, Conri C. Circulating markers of endothelial function in cardiovascular disease. Clin Chim Acta [Internet]. 2006 Jun [cited 2018 Jun 27];368(1–2):33–47. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16530177
131. Palella FJ, Phair JP. Cardiovascular disease in HIV infection. Curr Opin HIV AIDS [Internet]. 2011 Jul [cited 2017 Aug 7];6(4):266–71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21546831
132. Fan D, Takawale A, Lee J, Kassiri Z. Cardiac fibroblasts, fibrosis and extracellular matrix remodeling in heart disease. Fibrogenesis Tissue Repair [Internet]. 2012 [cited 2017 Jul 31];5(1):15. Available from: https://fibrogenesis.biomedcentral.com/articles/10.1186/1755-1536-5-15
133. Tomasek J, Gabbiani G, Hinz B. Myofibroblasts and mechano-regulation of connective tissue remodelling. Nat Rev [Internet]. 2002 [cited 2017 Aug 1]; Available from: http://search.proquest.com/openview/575848da8e43d7e5e6f2969357c2bcc5/1?pq-origsite=gscholar&cbl=27585
134. Baicu CF, Stroud JD, Livesay VA, Hapke E, Holder J, Spinale FG, et al. Changes in extracellular collagen matrix alter myocardial systolic performance. Am J Physiol - Hear Circ Physiol [Internet]. 2003 [cited 2017 Oct 4];284(1). Available from: http://ajpheart.physiology.org/content/284/1/H122
135. Heling A, Zimmermann R, Kostin S, Maeno Y. Increased expression of cytoskeletal, linkage, and extracellular proteins in failing human myocardium. Circulation [Internet]. 2000 [cited 2017 Jul 31]; Available from: http://circres.ahajournals.org/content/86/8/846.short
136. Iles L, Pfluger H, Phrommintikul A, Cherayath J, Aksit P, Gupta SN, et al. Evaluation of Diffuse Myocardial Fibrosis in Heart Failure With Cardiac Magnetic Resonance Contrast-Enhanced T1 Mapping. J Am Coll Cardiol [Internet]. 2008 Nov 1 [cited 2017 Jul 31];52(19):1574–80. Available from: http://circ.ahajournals.org/content/91/1/161.short
137. Frangogiannis NG. Regulation of the Inflammatory Response in Cardiac Repair. Rosenzweig A, editor. Circ Res [Internet]. 2012 Jan 6 [cited 2018 Jun 27];110(1):159–73. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22223212
138. Weber KT, Brilla CG. Pathological hypertrophy and cardiac interstitium. Fibrosis and renin- angiotensin-aldosterone system. Circulation [Internet]. 1991 Jun 1 [cited 2017 Jul 31];83(6):1849–65. Available from: http://circ.ahajournals.org/content/83/6/1849.short
139. Cartledge JE, Kane C, Dias P, Tesfom M, Clarke L, Mckee B, et al. Functional crosstalk between cardiac fibroblasts and adult cardiomyocytes by soluble mediators. Cardiovasc Res [Internet]. 2015 Mar 1 [cited 2018 Jun 27];105(3):260–70. Available from: https://academic.oup.com/cardiovascres/article-lookup/doi/10.1093/cvr/cvu264
140. Bohl S, Wassmuth R, Abdel-Aty H, Rudolph A. Delayed enhancement cardiac magnetic resonance imaging reveals typical patterns of myocardial injury in patients with various forms of non-ischemic heart disease. Int J [Internet]. 2008 [cited 2017 Jul 31]; Available from: http://link.springer.com/article/10.1007/s10554-008-9300-x
141. Anversa P, Hiler B, Ricci R, Guideri G, Olivetti G. Myocyte cell loss and myocyte hypertrophy in the aging rat heart. J Am Coll Cardiol [Internet]. 1986 Dec [cited 2017 Jul 31];8(6):1441–8. Available from: http://www.sciencedirect.com/science/article/pii/S0735109786803217
142. Anversa P, Nadal-Ginard B. Myocyte renewal and ventricular remodelling. Nature

[Internet]. 2002 Jan 10 [cited 2017 Jul 31];415(6868):240–3. Available from: https://www.nature.com/articles/415240a
143. Marijianowski M, Teeling P, Mann J. Dilated cardiomyopathy is associated with an increase in the type I/type III collagen ratio: a quantitative assessment. J Am [Internet]. 1995 [cited 2017 Jul 31]; Available from: http://www.sciencedirect.com/science/article/pii/0735109794005577
144. Tanaka M, Fujiwara H, Onodera T, Wu DJ, Hamashima Y, Kawai C. Quantitative analysis of myocardial fibrosis in normals, hypertensive hearts, and hypertrophic cardiomyopathy. Heart [Internet]. 1986 Jun 1 [cited 2017 Jul 31];55(6):575–81. Available from: http://heart.bmj.com/content/55/6/575.short
145. Schiffmann R, Kopp J, III HA, Sabnis S. Enzyme replacement therapy in Fabry disease: a randomized controlled trial. Jama [Internet]. 2001 [cited 2017 Jul 31]; Available from: http://jamanetwork.com/journals/jama/fullarticle/193887
146. Emoto R, Yokota Y, Miki T, Nomura H, Miki T, Usuki S, et al. [Prognosis of hypertrophic cardiomyopathy: echocardiographic and postmortem histopathologic study of 30 patients]. J Cardiol [Internet]. 1988 Sep [cited 2018 Jun 28];18(3):695–703. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3249285
147. Loring Z, Zareba W, McNitt S. ECG Quantification of Myocardial Scar and Risk Stratification in MADIT-II. Ann [Internet]. 2013 [cited 2017 Jul 31]; Available from: http://onlinelibrary.wiley.com/doi/10.1111/anec.12065/full
148. Aoki T, Fukumoto Y, Sugimura K, Oikawa M. Prognostic impact of myocardial interstitial fibrosis in non-ischemic heart failure. Circulation [Internet]. 2011 [cited 2017 Jul 31]; Available from: https://www.jstage.jst.go.jp/article/circj/75/11/75_CJ-11-0568/_article/-char/ja/
149. Yancy C, Jessup M, Bozkurt B, Butler J, Casey D. 2013 ACCF/AHA guideline for the management of heart failure. Circulation [Internet]. 2013 [cited 2017 Jul 31]; Available from: http://circ.ahajournals.org/content/circulationaha/early/2013/06/03/CIR.0b013e31829e8776.full.pdf
150. Ho J, Liu C, Lyass A, Courchesne P. Galectin-3, a marker of cardiac fibrosis, predicts incident heart failure in the community. J Am [Internet]. 2012 [cited 2017 Jul 31]; Available from: http://www.sciencedirect.com/science/article/pii/S0735109712023881
151. Passino C, Barison A, Vergaro G, Gabutti A. Markers of fibrosis, inflammation, and remodeling pathways in heart failure. Clin Chim [Internet]. 2015 [cited 2017 Jul 31]; Available from: http://www.sciencedirect.com/science/article/pii/S000989811400401X
152. Rooij E Van, Olson E. MicroRNA therapeutics for cardiovascular disease: opportunities and obstacles. Nat Rev Drug Discov [Internet]. 2012 [cited 2017 Jul 31]; Available from: http://search.proquest.com/openview/9e17a53f70c59698871a4a69b176a914/1?pq-origsite=gscholar&cbl=27580
153. Prockop D, Kivirikko K. Collagens: molecular biology, diseases, and potentials for therapy. Annu Rev Biochem [Internet]. 1995 [cited 2017 Jul 31]; Available from: http://www.annualreviews.org/doi/pdf/10.1146/annurev.bi.64.070195.002155
154. Roberts W, Siegel R, McManus B. Idiopathic dilated cardiomyopathy: analysis of 152 necropsy patients. Am J Cardiol [Internet]. 1987 [cited 2017 Jul 31]; Available from: http://www.sciencedirect.com/science/article/pii/0002914987906187
155. Brooks A, Schinde V, Bateman A, Gallagher P. Interstitial fibrosis in the dilated non-ischaemic myocardium. Heart [Internet]. 2003 [cited 2017 Jul 31]; Available from: http://heart.bmj.com/content/89/10/1255.short
156. Schalla S, Bekkers S, Dennert R. Replacement and reactive myocardial fibrosis in idiopathic dilated cardiomyopathy: comparison of magnetic resonance imaging with

right ventricular biopsy. Eur J [Internet]. 2010 [cited 2017 Jul 31]; Available from: http://onlinelibrary.wiley.com/doi/10.1093/eurjhf/hfq004/full
157. Vogel-Claussen J, Rochitte C, Wu K, Kamel I. Delayed enhancement MR imaging: utility in myocardial assessment. Radiographics [Internet]. 2006 [cited 2017 Jul 31]; Available from: http://pubs.rsna.org/doi/abs/10.1148/rg.263055047
158. Rehwald W, Fieno D, Chen E, Kim R, Judd R. Myocardial magnetic resonance imaging contrast agent concentrations after reversible and irreversible ischemic injury. Circulation [Internet]. 2002 [cited 2017 Aug 10]; Available from: http://circ.ahajournals.org/content/105/2/224.short
159. Parsai C, O'Hanlon R. Diagnostic and prognostic value of cardiovascular magnetic resonance in non-ischaemic cardiomyopathies. J [Internet]. 2012 [cited 2017 Jul 31]; Available from: https://jcmr-online.biomedcentral.com/articles/10.1186/1532-429X-14-54
160. Karamitsos TD, Francis JM, Myerson S, Selvanayagam JB, Neubauer S. The role of cardiovascular magnetic resonance imaging in heart failure. J Am Coll Cardiol [Internet]. 2009 Oct 6 [cited 2017 Aug 1];54(15):1407–24. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0735109709023729
161. Klein C, Nekolla S, Balbach T. The influence of myocardial blood flow and volume of distribution on late Gd-DTPA kinetics in ischemic heart failure. J Magn [Internet]. 2004 [cited 2017 Jul 31]; Available from: http://onlinelibrary.wiley.com/doi/10.1002/jmri.20164/full
162. Rathod RH, Powell AJ, Geva T, Ernst S, Swan L, Li W. Myocardial Fibrosis in Congenital Heart Disease. Circ J [Internet]. 2016 May 25 [cited 2017 Aug 21];80(6):1300–7. Available from: https://www.jstage.jst.go.jp/article/circj/80/6/80_CJ-16-0353/_article
163. White SK, Sado DM, Flett AS, Moon JC. Characterising the myocardial interstitial space: the clinical relevance of non-invasive imaging. Heart [Internet]. 2012 May 15 [cited 2017 Aug 10];98(10):773–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22422587
164. Ugander M, Bagi PS, Oki AJ, Chen B, Hsu L-Y, Aletras AH, et al. Myocardial Edema as Detected by Pre-Contrast T1 and T2 CMR Delineates Area at Risk Associated With Acute Myocardial Infarction. JACC Cardiovasc Imaging [Internet]. 2012 Jun [cited 2018 Jun 28];5(6):596–603. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22698528
165. Moon J, Messroghli D. Myocardial T1 mapping and extracellular volume quantification: a Society for Cardiovascular Magnetic Resonance (SCMR) and CMR Working Group of the. J [Internet]. 2013 [cited 2017 Aug 17]; Available from: https://jcmr-online.biomedcentral.com/articles/10.1186/1532-429X-15-92
166. Burt JR, Zimmerman SL, Kamel IR, Halushka M, Bluemke DA. Myocardial T1 Mapping: Techniques and Potential Applications. RadioGraphics [Internet]. 2014 Mar 1 [cited 2018 Jun 30];34(2):377–95. Available from: http://pubs.rsna.org/doi/10.1148/rg.342125121
167. Germain P, Ghannudi S El, Jeung M. Native T1 Mapping of the Heart–A Pictorial Review. Clin Med (Northfield Il) [Internet]. 2014 [cited 2017 Jul 31]; Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4251189/
168. Jellis C, Kwon D. Myocardial T1 mapping: modalities and clinical applications. Cardiovasc Diagn Ther [Internet]. 2014 [cited 2017 Jul 31]; Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3996234/
169. Arheden H, Saeed M, Higgins CB, Gao D-W, Bremerich J, Wyttenbach R, et al. Measurement of the Distribution Volume of Gadopentetate Dimeglumine at Echo-

planar MR Imaging to Quantify Myocardial Infarction: Comparison with 99m Tc-DTPA Autoradiography in Rats. Radiology [Internet]. 1999 Jun [cited 2018 Jun 30];211(3):698–708. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10352594
170. Kellman P, Wilson JR, Xue H, Ugander M, Arai AE. Extracellular volume fraction mapping in the myocardium, part 1: evaluation of an automated method. J Cardiovasc Magn Reson [Internet]. 2012 Sep 10 [cited 2018 Jun 27];14(1):63. Available from: http://jcmr-online.biomedcentral.com/articles/10.1186/1532-429X-14-63
171. Bandula S, Banypersad SM, Sado D, Flett AS, Punwani S, Taylor SA, et al. Measurement of Tissue Interstitial Volume in Healthy Patients and Those with Amyloidosis with Equilibrium Contrast-enhanced MR Imaging. Radiology [Internet]. 2013 Sep 1 [cited 2018 Jun 27];268(3):858–64. Available from: http://pubs.rsna.org/doi/10.1148/radiol.13121889
172. Lurz J, Luecke C, Lang D, Besler C. CMR–Derived Extracellular Volume Fraction as a Marker for Myocardial Fibrosis: The Importance of Coexisting Myocardial Inflammation. JACC Cardiovasc [Internet]. 2017 [cited 2017 Sep 18]; Available from: http://www.sciencedirect.com/science/article/pii/S1936878X17302541
173. Bloch F. Nuclear Induction. Phys Rev [Internet]. 1946 Oct 1 [cited 2017 Dec 1];70(7–8):460–74. Available from: https://link.aps.org/doi/10.1103/PhysRev.70.460
174. Currie S, Hoggard N, Craven IJ, Hadjivassiliou M, Wilkinson ID. Understanding MRI: basic MR physics for physicians. Postgrad Med J [Internet]. 2013 Apr 1 [cited 2018 Jun 28];89(1050):209–23. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23223777
175. Basic MRI Physics - wikidoc [Internet]. [cited 2018 Jun 25]. Available from: http://wikidoc.org/index.php/Basic_MRI_Physics
176. Nacif M, Turkbey E, Gai N. Myocardial T1 mapping with MRI: comparison of look-locker and MOLLI sequences. J Magn [Internet]. 2011 [cited 2017 Jul 31]; Available from: http://onlinelibrary.wiley.com/doi/10.1002/jmri.22753/full
177. Piechnik S, Ferreira V. Shortened Modified Look-Locker Inversion recovery (ShMOLLI) for clinical myocardial T1-mapping at 1.5 and 3 T within a 9 heartbeat breathhold. J [Internet]. 2010 [cited 2017 Jul 31]; Available from: https://jcmr-online.biomedcentral.com/articles/10.1186/1532-429X-12-69
178. Roujol S, Weingärtner S, Foppa M, Chow K, Kawaji K. Accuracy, precision, and reproducibility of four T1 mapping sequences: a head-to-head comparison of MOLLI, ShMOLLI, SASHA, and SAPPHIRE. Radiology [Internet]. 2014 [cited 2017 Jul 31]; Available from: http://pubs.rsna.org/doi/abs/10.1148/radiol.14140296
179. Dass S, Suttie JJ, Piechnik SK, Ferreira VM, Holloway CJ, Banerjee R, et al. Myocardial tissue characterization using magnetic resonance noncontrast t1 mapping in hypertrophic and dilated cardiomyopathy. Circ Cardiovasc Imaging [Internet]. 2012 Nov 1 [cited 2017 Jul 31];5(6):726–33. Available from: http://circimaging.ahajournals.org/content/5/6/726.short
180. Marckmann P, Skov L, Rossen K, Dupont A. Nephrogenic systemic fibrosis: suspected causative role of gadodiamide used for contrast-enhanced magnetic resonance imaging. J [Internet]. 2006 [cited 2017 Jul 31]; Available from: http://jasn.asnjournals.org/content/17/9/2359.short
181. Sharma S, Adrogue J, Golfman L, Uray I, Lemm J. Intramyocardial lipid accumulation in the failing human heart resembles the lipotoxic rat heart. FASEB J [Internet]. 2004 [cited 2017 Jul 31]; Available from: http://www.fasebj.org/content/18/14/1692.short
182. Rutherford E, Talle M, Mangion K, Bell E. Defining myocardial tissue abnormalities in end-stage renal failure with cardiac magnetic resonance imaging using native T1 mapping. Kidney Int [Internet]. 2016 [cited 2017 Jul 31]; Available from: http://www.sciencedirect.com/science/article/pii/S0085253816302988

183. Nakamori S, Dohi K, Ishida M, Goto Y. Native T1 Mapping and Extracellular Volume Mapping for the Assessment of Diffuse Myocardial Fibrosis in Dilated Cardiomyopathy. JACC Cardiovasc [Internet]. 2017 [cited 2017 Jul 31]; Available from: http://www.sciencedirect.com/science/article/pii/S1936878X1730400X
184. Kawel N, Nacif M, Zavodni A. T1 mapping of the myocardium: intra-individual assessment of the effect of field strength, cardiac cycle and variation by myocardial region. J [Internet]. 2012 [cited 2017 Jul 31]; Available from: https://jcmr-online.biomedcentral.com/articles/10.1186/1532-429X-14-27
185. Everett RJ, Newby DE, Jabbour A, Fayad ZA, Dweck MR. The Role of Imaging in Aortic Valve Disease. Curr Cardiovasc Imaging Rep [Internet]. 2016 Jul 7 [cited 2018 May 19];9(7):21. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27375833
186. Jerosch-Herold M, Sheridan D. Cardiac magnetic resonance imaging of myocardial contrast uptake and blood flow in patients affected with idiopathic or familial dilated cardiomyopathy. Am J [Internet]. 2008 [cited 2017 Jul 31]; Available from: http://ajpheart.physiology.org/content/295/3/H1234.short
187. van der Geest RJ, Buller VG, Jansen E, Lamb HJ, Baur LH, van der Wall EE, et al. Comparison between manual and semiautomated analysis of left ventricular volume parameters from short-axis MR images. J Comput Assist Tomogr [Internet]. [cited 2018 May 19];21(5):756–65. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9294570
188. Sado D, Flett A, Banypersad S, White S. Cardiovascular magnetic resonance measurement of myocardial extracellular volume in health and disease. Heart [Internet]. 2012 [cited 2017 Jul 31]; Available from: http://heart.bmj.com/content/early/2012/08/29/heartjnl-2012-302346.short
189. Miller CA, Naish JH, Bishop P, Coutts G, Clark D, Zhao S, et al. Comprehensive Validation of Cardiovascular Magnetic Resonance Techniques for the Assessment of Myocardial Extracellular Volume. Circ Cardiovasc Imaging [Internet]. 2013 May 1 [cited 2017 Jul 31];6(3):373–83. Available from: http://circimaging.ahajournals.org/content/circcvim/early/2013/04/03/CIRCIMAGING.112.000192.full.pdf
190. Karamitsos TD, Piechnik SK, Banypersad SM, Fontana M, Ntusi NB, Ferreira VM, et al. Noncontrast T1 mapping for the diagnosis of cardiac amyloidosis. JACC Cardiovasc Imaging [Internet]. 2013 Apr [cited 2018 May 19];6(4):488–97. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23498672
191. Sado DM, White SK, Piechnik SK, Banypersad SM, Treibel T, Captur G, et al. Identification and assessment of Anderson-Fabry disease by cardiovascular magnetic resonance noncontrast myocardial T1 mapping. Circ Cardiovasc Imaging [Internet]. 2013 May 1 [cited 2018 May 19];6(3):392–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23564562
192. Sparrow P, Messroghli DR, Reid S, Ridgway JP, Bainbridge G, Sivananthan MU. Myocardial T1 mapping for detection of left ventricular myocardial fibrosis in chronic aortic regurgitation: pilot study. AJR Am J Roentgenol [Internet]. 2006 Dec [cited 2018 May 19];187(6):W630-5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17114517
193. Lee S-P, Lee W, Lee JM, Park E-A, Kim H-K, Kim Y-J, et al. Assessment of diffuse myocardial fibrosis by using MR imaging in asymptomatic patients with aortic stenosis. Radiology [Internet]. 2015 Feb [cited 2018 May 19];274(2):359–69. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25251584
194. Yi CJ, Wu CO, Tee M, Liu C-Y, Volpe GJ, Prince MR, et al. The association between cardiovascular risk and cardiovascular magnetic resonance measures of fibrosis: the Multi-Ethnic Study of Atherosclerosis (MESA). J Cardiovasc Magn Reson [Internet].

2015 Feb 12 [cited 2018 May 19];17(1):15. Available from: http://jcmr-online.com/content/17/1/15
195. Taylor AJ, Salerno M, Dharmakumar R, Jerosch-Herold M. T1 Mapping. JACC Cardiovasc Imaging [Internet]. 2016 Jan [cited 2018 May 19];9(1):67–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26762877
196. Perea RJ, Ortiz-Perez JT, Sole M, Cibeira MT, de Caralt TM, Prat-Gonzalez S, et al. T1 mapping: characterisation of myocardial interstitial space. Insights Imaging. 2015;6(2):189–202.
197. Anderson DW, Virmani R, Reilly JM, O'Leary T, Cunnion RE, Robinowitz M, et al. Prevalent myocarditis at necropsy in the acquired immunodeficiency syndrome. J Am Coll Cardiol [Internet]. 1988 Apr [cited 2018 Jun 30];11(4):792–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3351145
198. Cerrato E, D'Ascenzo F, Biondi-Zoccai G, Calcagno A, Frea S, Grosso Marra W, et al. Cardiac dysfunction in pauci symptomatic human immunodeficiency virus patients: a meta-analysis in the highly active antiretroviral therapy era. Eur Heart J [Internet]. 2013 May 1 [cited 2018 Jan 24];34(19):1432–6. Available from: http://www.scopus.com/inward/record.url?eid=2-s2.0-84877005066&partnerID=40&md5=6bbb4f8d325d5974f9042927c01a2096
199. Post WS, Budoff M, Kingsley L, Palella FJ, Witt MD, Li X, et al. Associations Between HIV Infection and Subclinical Coronary Atherosclerosis. Ann Intern Med [Internet]. 2014 Apr 1 [cited 2018 Jun 30];160(7):458. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24687069
200. Rider OJ, Asaad M, Ntusi N, Wainwright E, Clutton G, Hancock G, et al. HIV is an independent predictor of aortic stiffness. J Cardiovasc Magn Reson [Internet]. 2014 Aug 16 [cited 2018 Jun 30];16(1):57. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25187084

Appendices

1. Appendix A

Predictors of Diffuse Myocardial Fibrosis in HIV Infected Persons: A Multiparametric Cardiovascular Magnetic Resonance Study

You are invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your doctor, if you wish. This leaflet will tell you the purpose of the study, what will happen to you when you take part and gives you detailed information about the conduct of the study.

Ask us if there is anything that is not clear or if you would like more information. Thank you for reading this.

What is the purpose of the study?

Patients with HIV infection have increased scarring in the heart. Our previous research has shown that the heart muscle of patients with HIV has increased scar, which is even higher in patients who have previously been treated for tuberculosis of the heart. However, the causes of increased scar in the hearts of patients with HIV are unknown. The purpose of the study is to use cardiovascular magnetic resonance imaging (MRI) to study the nature and frequency of heart and blood vessel disease in people with these conditions, to understand mechanisms of disease and the causes of increased scarring in the muscle of the heart.

If you take part in this study, you will be seen at a visit, where you will be examined, have a resting electrocardiogram (ECG), and be asked to provide us with a blood sample. We will also examine the function of your heart and blood vessels with an MRI scan. You will not be asked to take any additional long-term pill or alter your regular medication in any way.

Why have I been invited?

You have been invited because you suffer from HIV. A minimum of 50 participants will be invited to participate in the patient group of this study.

Do I have to take part?

It is up to you to decide whether to take part. If you decide to take part, you are free to withdraw consent at any time without giving a reason. Your decision will not affect the standard of care you receive. If you decide that you no longer wish to continue with the study, we would still retain any data already obtained from you up to the point of your withdrawal.

What would happen to me if I take part?

You would attend a visit that will last between 1.5 and 2 hours. At this visit, we will ask some general questions about your health and regular medication. Next, we will do a brief examination that includes

measurement of your pulse, blood pressure, weight and height. An ECG trace of your heart's electrical impulse will be done. After that, we will take a blood sample, and scan your heart and blood vessels using non-invasive cardiac MRI. If your ultrasound or MRI scan suggests abnormalities, we will advise your doctors to refer you to a cardiologist for review.

Below, all the above-mentioned tests are discussed in a little bit more detail:

Clinical assessment

The assessment will start by asking you a set of questions about your health and previous medical conditions, using a structured questionnaire. The physical exam will include measurement of your pulse, blood pressure, weight and height, as well as an examination of the cardiovascular and rheumatological systems.

Blood tests (5-10 minutes)

If you haven't had a blood test for kidney function in the last six months, or if we look at your medical history and judge that you need a more recent test, we will take a small blood sample from you. This is to check that your kidneys are functioning well and can process the contrast agents we use in some of the scans. If your blood tests show reduced kidney function, you may not be able to take part in some of this study. We will also take 20mls of blood from you, to measure the levels of certain blood-borne substances and markers of inflammation.

The heart MRI scan (approximately 60 minutes)

The MRI scan of your heart will be the most important part of this study. MRI scans are painless but involve the use of a strong magnetic field, so if you have any of the following, you would not be suitable for a scan, and would not be able to take part in this study:

- a permanent pacemaker
- metal clips in blood vessels of the brain
- an injury to the eye involving fragments of metal
- insulin pump
- shrapnel injuries
- other metal or electronic implants affected by the magnetic field
- neurostimulators
- cochlear implant

The MRI scanner is shaped like a polo mint, the hole inside measuring about 60-70 centimetres wide, with a table that slides in and out. You will be asked to change into a hospital gown and to lie still on your back on the table, while your heart is scanned. You will also be asked to breathe in and out and hold your breath for several seconds for some of the scans. Pictures of the heart are created using a magnetic field, radio waves and computers. When images are being taken, the MRI scanner makes a loud noise, and you will be provided with earphones to protect your ears. It is important that you lie still for the duration of the scan.

Use of contrast dye (gadolinium)

To evaluate fully the blood circulation and your heart muscle for scarring and tissue characteristics we shall inject some contrast dye, called gadolinium, through a drip in your arm.

The ECG (5 minutes)

We will take an electrocardiogram (ECG) of your heart. An ECG is a tool that uses surface electrodes on certain points on your chest and arms to monitor the electrical properties of your heart. The ECG is a non-invasive test and does not cause any harm.

Optional second heart MRI

Participants who are agreeable may be offered a second heart MRI scan, which is optional. These participants may be invited to return for a second CMR study, at 3-5 years after the baseline scan. The purpose of the second scan is to assess (1) the effect of treatment on heart and blood vessel structure and function, (2) the natural history of heart and blood vessel involvement in these diseases, and (3) to study cardiovascular outcomes in these clinical entities. It is important to note that the second MRI scan will be identical to the first, and there no increased or additional risk from this.

Follow-up at 3 to 5 years

Participants will be followed up telephonically at 3-5 years to check on their health and whether they have developed any cardiovascular complications related to their condition.

What about travel expenses?

We will reimburse travel expenses to and from the hospital. Lunch will be provided or reimbursed.

What will I have to do, if I agree to take part in this study?

1. Attend a visit at Groote Schuur Hospital for the assessment, blood tests and for the scans.
2. Consent to taking part in this study by signing a form.
3. Undergo the procedures as described above.

Are there any other possible risks from taking part?

The scanning is done using an MRI scanner which is also used routinely in clinical practice to acquire images of various body parts. MRI scans are safe, non-invasive and do not involve any ionising

radiation (X-rays). Some people find the space limitation in the scanner uncomfortable, but you will be given a chance to see the scanner to make sure that you are comfortable in it before the study starts. The scan is noisy and we provide headphones to protect your ears. The whole time that you are in the scanner you will be given a buzzer which you will be able to use at any time if you wish to stop the study. As the scanner consists of a powerful magnet, it may attract certain metallic objects. You must not have a scan if you have had metallic objects or medical devices (e.g. pacemaker) inserted into your body during an operation. While MRI is safe in pregnancy, because this is a research study, as a precaution we advise you to tell us if there is any chance you might be pregnant. The doctor or radiographer will go through a list of possible risks with you before you go into the scanner.

In the unlikely event of us seeing any structural abnormalities on your MRI scan, a member of our research team will discuss the implications with you, and, with your permission, your doctor may be notified. However, it is important to note that we do not carry out scans for diagnostic purposes, and therefore these scans are not a substitute for a clinical appointment. Rather, our scans are intended for research purposes only. Some people find having a drip in their arm uncomfortable and there can be bruising at the site of needle entry. Our staff is trained in drip insertion and we will make sure you are as comfortable as possible.

Gadolinium, the dye used for the MRI scans, has been in clinical use for over 20 years. As the dye is being injected, some people report a sensation of warmth at the injection site. It is unusual to feel pain, and in this case, we would stop the injection immediately. Rarely, some people feel slightly nauseous or have a metallic taste following the injection, but vomiting is exceedingly rare. Occasionally, people have developed a rash; however, severe allergic reactions are very rarely. Again, this dye is injected through a drip in the arm. There is no pain associated with the injection at the site. There is a small risk (less than 1 in 1000) of an allergic reaction to the dye.

ECG and ultrasound are safe, non-invasive tests, with no known serious risks/harm. Rarely, individuals having either test may develop an allergic reaction to placement of electrodes that results in a mild rash. This rash disappears in a few days without any treatment.

It is important to note that in a large-volume MRI centre like Groote Schuur hospital, where our experienced staff has been doing MRI scans for many years, the risk of harm from the MRI and other tests is exceedingly small.

What are the possible benefits?

There is no direct benefit for you as an individual taking part in this study. We hope that by studying people with your condition using cardiac MRI, we may be able to improve understanding of this condition and help to inform screening/treatment of future patients.

What happens when the research study stops?

The end of the study will not affect the care you receive from your doctors. The end of the study will mark the official end of your participation in this project. Copies of any publications connected to this study will be available on request from Prof. Ntusi.

Will my taking part in the study be kept confidential?

Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. If you take part in the study, some of the data collected from the study would be looked at by authorised persons from the University of Cape Town, to check that the study is being carried out

correctly. All investigators have a duty of confidentiality to you as a research participant, and nothing that could reveal your identity would be disclosed outside the research site. The data collected from the study will be recorded anonymously and you would not be identifiable from this. Blood samples will be coded, and the identity of the bloods will be kept confidential. In the publication, there will be no identifying details.

What if relevant new information becomes available?

Sometimes, we (the study investigators) get new information about the procedures being studied. If this happens, one of us will tell you and discuss whether you should continue in the study. If there is enough evidence to suggest you may be harmed from participating in this study, the study could be stopped.

Unexpected findings on your scan

In the unlikely event of us seeing any structural abnormalities on your MRI scan, a designated clinical specialist will discuss the implications with you and may arrange for further investigations as necessary. However, it is important to note that we do not carry out scans for diagnostic purposes, and therefore these scans are not a substitute for a clinical appointment. Rather, our scans are intended for research purposes only. So, if we find anything unusual, it would be appropriate for us to contact your GP/specialist so that they can arrange on-going clinical care for you. But we would only do this after we and the specialist had discussed your options and gained your permission.

What will happen if I don't want to carry on with the study?

You are free to withdraw from the study at any time. Anonymised data will be kept till the point you choose to end your participation in the study.

What will happen to any samples I give?

All samples will be retained in a secure environment for future analysis and will be stored in an anonymous format. With permission from the ethics committee, the samples will be stored for 5 years and may be used in future research as our understanding of heart and blood vessel function grows. Should we wish to utilise samples of blood obtained from you for new tests, we will contact you to seek your approval for performance of these additional tests. Samples will be destroyed by the research team when ethical favourable opinion lapses.

What will happen to the results of the research study?

We anticipate that the results will be published in a scientific journal for the benefit of the wider medical community. However, individual patients will not be identified in any publication and your personal and clinical details will remain strictly confidential. Any scientific publications arising from the study will be available on request to all participants. You would have no legal right to a share of any profits that may arise from the research.

Will your test results be shared with you?

We will show you the images we acquire from the ultrasound and MRI scans when we finish performing the scans. The results of the other tests will only be available on publication of the results. If however, results of any of any of the tests are grossly abnormal, we will contact you to discuss these with you before suggesting a course of action and contacting your doctor/specialist.

Who is organising and funding the research?

The study organised and conducted by researchers from the University of Cape Town. The researchers have applied for an investigator-led grant from the National Research Foundation of South Africa.

What happens if I get hurt taking part in this study?

The University of Cape Town (UCT) has insurance cover for the event that research-related injury or harm results from your participation in the trial. The insurer will pay all reasonable medical expenses in accordance with the South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI) in the event of an injury or side effect resulting directly from your participation in the trial. You will not be required to prove fault on the part of the University.

The University will not be liable for any loss, injuries and/or harm that you may sustain where the loss is caused by

- The use of unauthorised medicine or substances during the study
- Any injury that results from you not following the protocol requirements or the instructions that the study doctor may give you
- Any injury that arises from inadequate action or lack of action to deal adequately with a side effect or reaction to the study medication
- An injury that results from negligence on your part

[Researchers must bear in mind that it is unacceptable to impose a burden on participants who may not recognize symptoms or have the ready means to take action.]

"By agreeing to participate in this study, you do not give up your right to claim compensation for injury where you can prove negligence, in separate litigation. In particular, your right to pursue such a claim in a South African court in terms of South African law must be ensured. Note, however, that you will usually be requested to accept that payment made by the University under the SA GCP guideline 4.11 is in full settlement of the claim relating to the medical expenses. "

An injury is considered trial-related if, and to the extent that, it is caused by study activities. You must notify the study doctor immediately of any side effects and/or injuries during the trial, whether they are research-related or other related complications.

UCT reserves the right not to provide compensation if, and to the extent that, your injury came about because you chose not to follow the instructions that you were given while you were taking part in the study. Your right in law to claim compensation for injury where you prove negligence is not affected. Copies of these guidelines are available on request.

Who has reviewed the study?
The University Of Cape Town Faculty Of Health Sciences Human Research Ethics Committee has reviewed and approved the study.

Further information and contact details
Should you wish to know more about any aspects of this study, please contact Prof. Ntusi at 021 406 6200.

Should you have any concerns regarding your rights or welfare as a research participant, please contact the University Of Cape Town Faculty Of Health Sciences Human Research Ethics Committee at 021 406 6626.

2. Appendix B

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6526
Email: shurette.thomas@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

06 March 2017

HREC REF: 140/2017

Prof N Ntusi
Medicine
J-floor, OMB

Dear Prof Ntusi

PROJECT TITLE: PREDICTORS OF DIFFUSE MYOCARDIAL FIBROSIS IN HIV-INFECTED PERSONS: A MULTIPARAMETRIC CARDIOVASCULAR MAGNETIC RESONANCE STUDY (MASTER OF SCIENCE CANDIDATE - H SAAD) SUB-STUDY LINKED TO 660/2015

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee.

Before formal approval can be granted, please address the following issue/s raised:

1. Please supply a simple informed consent document for HIV non-infected participants.
2. Please also justify the sample size of 25 per arm.
3. Please provide the annual progress report for study 660/2015.

Please note that no research may occur without formal written HREC approval.

Please quote the HREC reference number in all your correspondence.

The HREC acknowledge that the student, Hadil Saad will also be involved in this study.

Yours sincerely

Signature Removed

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

3. Appendix C

CONSENT FORM

Study Full Title	Predictors of Diffuse Myocardial Fibrosis in HIV Infected Persons: A Multiparametric Cardiovascular Magnetic Resonance Study
Patient ID	
Researchers	Prof. Ntobeko Ntusi and Dr Hadil Saad

	I agree	I disagree
I confirm that I have read and understand the information sheet for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.		
I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.		
I understand that relevant sections of my medical notes and data collected during the study may be looked at by authorized individuals from the University of Cape Town, where it is relevant to my taking part in this research.		
I understand why blood samples are being taken, how the samples will be collected, that giving samples for this research is voluntary and that I am free to withdraw my approval for use of the sample at any time without giving a reason and without my medical treatment or legal rights being affected. I understand that any data collected from the analyses of the samples will be retained for use in the results of the research study		
I give permission for the research team to store my blood samples for up to 5 years before destroying them.		
I agree to donate blood samples taken for the purpose of the research study to the University of Cape Town. If a commercial product were developed as a result of this study, I will not profit financially from such a product.		
I understand that my doctor will, with my permission, be informed of the results of medical tests performed as part of the research, which are important for my health care.		
I understand that I may receive a phone call from the study investigators at 3 to 5 years after my scan to ask about my health.		
I also understand that I may be invited to return for a second MRI scan, which is optional.		
I agree to being contacted in the future to ask if I am interested in future related studies.		

Name of participant	Signature	Date
Name of person taking consent	Signature	Date