

**DETECTING SUBCLINICAL ANTHRACYCLINE THERAPY RELATED CARDIAC  
DYSFUNCTION IN LOW INCOME COUNTRY (SATRACD STUDY)**

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- 1: Zhang WZ, Azibani F, Sliwa K. Subclinical anthracycline therapy-related cardiac Dysfunction: an ignored stage B heart failure in an African population. *Cardiovascular journal of Africa*. 2020;31(5):262-6.
- 2: Zhang W, Azibani F, Okello E, Kayima J, Walusansa V, Orem J, et al. Rational and design of SATRACD study: detecting subclinical anthracycline therapy related cardiac dysfunction in low income country. *African health sciences*. 2021;21(2):647-54.
- 3: Zhang W, Azibani F, Okello E, Kayima J, Sinabulya I, Leeta J, et al. Clinical characterization, cardiovascular risk factor profile and cardiac strain analysis in a Uganda cancer population: The SATRACD study. *PLoS ONE*. 2021;16(4):e0249717.
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As the first author for all the papers, I substantially contributed to the conception and design of the work; the acquisition, analysis, and interpretation of data for the work; drafting the work and revising it; final approval of the version to be published; agreement to be accountable for all aspects of the work. These roles apply to all the papers listed above.

## **Dedication**

I dedicate this thesis to the loving Lord Jesus Christ, who is the light to my path, my dear husband, Charles, my lovely children Jimmy and Victoria for their patience and support, my beloved parents, sisters and brothers in Uganda Chinese Christian Church for the endless prayers in God and my three supervisors, Karen, Ferial and Emmy for their mentorship.

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## Thesis Abstract

### Detecting subclinical anthracycline therapy related cardiac dysfunction in low income country (SATRACD study)

Wanzhu Zhang

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**Introduction:** Anthracycline therapy-related cardiac dysfunction (ATRCD) is the most common chemotherapy-induced cardiovascular toxicity. It begins with subclinical myocardial cell injury that can be detected using speckle tracking echocardiography (STE), together with Troponin-I. Limited availability of STE in Uganda posts challenges in detecting subclinical ATRCD. Anthracycline can also affect cancer survivors' cardiovascular health through altering patients' lipid homeostasis. This PhD project aims to describe the incidence and predictors of subclinical ATRCD, assess the accuracy of simple echocardiographic parameters on detecting subclinical ATRCD and investigate the lipoprotein subfractions change after anthracycline therapy.

**Methods and results:** Two hundred seven cancer patients who were scheduled for anthracycline based chemotherapy were recruited and followed up to 6 months after ending chemotherapy. Patients' clinical characteristics, laboratory tests, electrocardiogram and echocardiographic data were collected at the baseline and at each follow up visits. Among the 207 patients, 178 (86.0%) were female, with a median age of 42 years. The cumulative incidence of subclinical and clinical ATRCD were 35.0% and 8.8% respectively at the 6 months after ending the therapy. No factor was found to predict subclinical ATRCD in multivariable model. The development of clinical ATRCD associated with HIV infection and development of subclinical ATRCD at the end of anthracycline therapy. The reduction of mitral annular plane systolic excursion ( $\Delta$ MAPSE)  $\geq$  2mm or reduction of mitral annular peak systolic velocity ( $\Delta$  S')  $\geq$  0.5cm/s from the baseline defined subclinical ATRCD with fairly good accuracy. Very low density lipoprotein subfraction increased and mean low density lipoprotein particle size decreased following anthracycline therapy.

**Conclusion:** There is high incidence of subclinical ATRCD in Uganda cancer patients. Cardiac surveillance at baseline and ending of anthracycline therapy is essential to identify subclinical ATRCD patients who are at high risk of developing clinical ATRCD, particular in HIV positive patients. The conventional echocardiographic parameters  $\Delta$ MAPSE and  $\Delta$ S' may be used to screen subclinical ATRCD in resource limited settings. Anthracycline changes lipoprotein subfraction to more atherogenic pattern. Further studies are needed to explore more on its role on lipid metabolism.

## **Overview of The Thesis**

In this PhD thesis, I present the research project in the field of cardio-oncology. The specific topic is around the cardiovascular side effect of a commonly used the chemotherapy regimen- anthracycline. The summaries of each chapter are listed below:

Chapter one: It is a comprehensive literature review which covers wide topics from the introduction of anthracycline family, the concept of anthracycline therapy related cardiac dysfunction (ATRCD) to the emerging knowledge of the subclinical ATRCD and the novel technology and guidelines to detect it. This chapter also summarizes the current cardiac care of anthracycline treated cancer patients in Africa, aiming to highlight the knowledge gaps and challenges in detecting, preventing and treating ATRCD in Uganda. Finally, it discusses briefly on the new concern regarding anthracycline 's impact on cardiovascular healthy- dyslipidemia.

Chapter two: Base on chapter one's introduction, chapter two states the current problems in Uganda and the rational, aims, hypothesis and the four objectives of the SATRACD study.

Chapter three: In this methodology chapter, I describe the main study design, patients' enrollment, follow up and data collection protocols.

Chapter four: In this chapter, I present and discuss patients' baseline (pre chemotherapy) data, aim to describe the prevalence of pre-existing cardiovascular risks, baseline cardiac strain pattern and the associations between the two. I found hypertension, obesity and HIV infection are the three most prevalent cardiovascular risk factors in the study population and obesity is associated with suboptimal left ventricular global longitudinal strain (GLS) at the baseline.

Chapter five: In this chapter, I am able to report the cumulative incidence of subclinical and clinical ATRCD using the longitudinal data of the project. I found a high incidence of ATRCD,

of which, majority are subclinical and free of heart failure symptoms. I further discuss the results of competing risk regression analysis aim to identify the predictors ATRCD and conclude that echocardiographic assessment at the baseline and at the end of anthracycline therapy are essential to identify the high risk patients, prevent the development of more severe form of cardiac dysfunction. This may be more important for HIV infected cancer patients. The work in chapter five fulfills study objective one and two.

Chapter six: This chapter presents a sub data analysis aiming to assess the roles of the two conventional echocardiographic parameters- reduction of mitral annular plane systolic excursion ( $\Delta$ MAPSE) and reduction of mitral annular peak systolic velocity ( $\Delta$ S') on detecting subclinical ATRCD, using GLS as a gold standard test. The results showed fairly good accuracy and high negative predictive values for both parameters, indicating they may serve as screening tools for subclinical ATRCD when speckle tracking echocardiography is not available. This sub analysis fulfills study objective three.

Chapter seven: In this chapter, I present a pilot study aiming to investigate the changes of lipoprotein subfractions after anthracycline therapy in breast cancer patients with low baseline cardiovascular risk. The result showed increased very low density lipoprotein (VLDL) subfraction, decreased mean low density lipoprotein (LDL) size and a shift of LDL subfractions pattern. I discuss the remarkable findings of the study which are able to generate more research questions, at the same time, note the limitation of the pilot study. This pilot study fulfills study objective four.

Chapter eight: This is the final chapter summarizes the findings and conclusions of the entire project, discusses the contribution of the research to the cardio-oncology services in Uganda and its future prospective.

## List of Abbreviations

CTRCD	Cancer therapy related cardiac dysfunction
ATRCD	Anthracycline therapy related cardiac dysfunction
HF	Heart Failure
NHL	non-Hodgkin's lymphoma
HL	Hodgkin's lymphoma
HCC	Hepatocellular carcinoma
HT	Hypertension
DM	Diabetes mellitus
CKD	Chronic kidney disease
LBBB	Left bundle branch block
BP	Blood pressure
BMI	Body mass index
BSA	Body surface area
ECG	Electrocardiogram
ECHO	Echocardiography
2D	Two dimensional
STE	Sparkle tracing echocardiography
TDI	Tissue Doppler Image
LV	Left ventricle
LA	Left atrium

LVEF	Left ventricular ejection fraction
GLS	Global longitudinal strain
MAPSE	Mitral annular plane systolic excursion
TAPSE	Tricuspid annular plane systolic excursion
S'	Mitral annular peak systolic velocity
SV	Stroke volume
CO	Cardiac output
CI	Cardiac index
LVOT	Left ventricular outflow track
LVEDV	Left ventricular end-diastolic volume
LVESV	Left ventricular end-systolic volume
ROI	Region of interest
AVC	Aortic valve closure
Top2	Topoisomerase II
cGMP	cyclic guanosine monophosphate
TnT	Troponin T
TnI	Troponin I
BNP	Brain-type natriuretic peptide
NT-proBNP	N-terminal pro-BNP
hsCRP	High-sensitivity C reactive protein
MPO	Myeloperoxidase

CM	Chylomicron
VLDL	Very low density lipoprotein
IDL	Intermediate density lipoprotein
LDL	Low density lipoprotein
HDL	High density lipoprotein
ASE	American Society of Echocardiography
EAE	European Association of Echocardiography
ESC	European Society of cardiology
ICOS	International Cardio-Oncology Society
UCI	Uganda Cancer Institute
UHI	Uganda Heart Institute
LMICs	Low-middle income countries
SD	Standard deviation
POR	Prevalence Odds Ratio
HR	Hazard Ratio
AUC	Area under the curve
PPV	Positive predictive value
NPV	Negative predictive value

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## **Chapter 1: Introduction: Background and review of literature**

### **1.0 Background**

Anthracyclines (daunomycin, doxorubicin, epirubicin, idarubicin) are a family of potent antineoplastic drugs which have been used to treat various types of solid and hematological malignancies. Anthracyclines are classified as antineoplastic antibiotics which bind to DNA at different levels, affecting cell replication and interfere with every phase of the cell cycle (Nadia P et al., 2008). “Doxorubicin and daunomycin are the more commonly used anthracyclines in clinical practice. They are administered only by intravenous infusion and metabolized by the liver. Their main route of excretion is through bile, while urinary elimination accounts for approximately 1/6 of the total amount (Nadia P et al., 2008).” Like many antineoplastic drugs, patients who receive anthracycline therapy may experience some of the transient side effects, such as myelosuppression, mucositis and hair loss. However, anthracycline therapy induced cardiotoxicity may be permanent and progressive (Nadia P et al., 2008). This dose-dependent anthracycline therapy related cardiac dysfunction (ATCRD) was first described in 1971 in a cohort of 67 patients treated with adriamycin (doxorubicin) for a variety of tumors (Middleman et al., 1971). The clinical significance of anthracycline cardiotoxicity is growing with increasing cancer survivors worldwide.

### **1.1 Definition and classification of ATRCD**

ATRDC is diagnosed by echocardiography (ECHO) with a definition of “decrease in the left ventricular ejection fraction (LVEF) of >10 percentage points, to a value <50% (normal reference value for two-dimensional (2D) ECHO). LVEF decrease may be further categorized as symptomatic or asymptomatic, or with regard to reversibility (Plana et al., 2014):

- Reversible: to within 5 percentage points of baseline

- Partially reversible: improved by  $\geq 10$  percentage points from the nadir but remaining  $> 5$  percentage points below baseline
- Irreversible: improved by  $< 10$  percentage points from the nadir and remaining  $> 5$  percentage points below baseline
- Indeterminate: patient not available for re-evaluation”

Anthracycline toxicity can be acute, early, or late onset. Acute toxicity which develops in 1% of patients immediately after infusion, is uncommon and generally reversible (Zamorano et al., 2016). Early effects occur within the first year of treatment, while late effects manifest after several years (median of 7 years after treatment) (Steinherz et al., 1991, Von Hoff DD, 1979). Early and late onset cardiac dysfunction are more likely to be irreversible (Von Hoff DD, 1979).

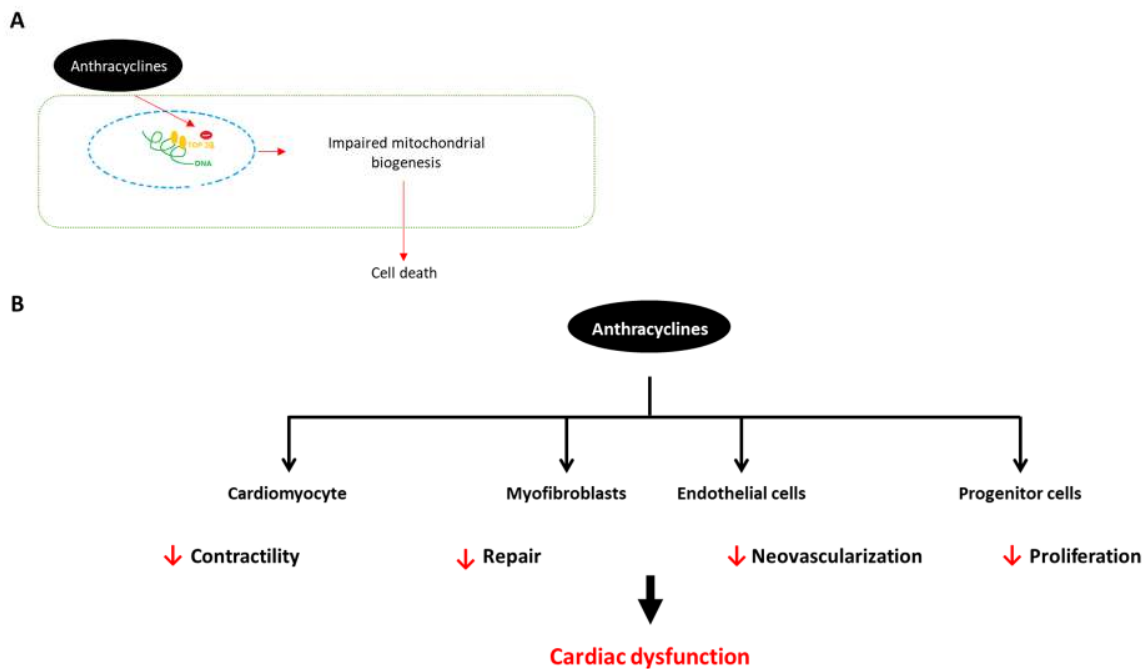
According to the American College of Cardiology and American Heart Association guidelines, patients who received cardio toxic agents are considered at stage A heart failure (HF) (Yancy et al., 2013, Heidenreich et al., 2022). This identifies patients who are at a high risk for developing HF with no evidence of cardiac structural or biomarker abnormalities (Heidenreich et al., 2022). Moreover, Stage B refers to patients with cardiac structural or biomarker abnormalities but who have never developed symptoms of HF. Stage C denotes patients with current or previous symptoms of HF associated with underlying structural heart disease and Stage D designates the patient with end-stage disease who requires specialized treatment strategies such as mechanical circulatory support, continuous inotropic infusions, cardiac transplantation, or hospice care. This approach to the classification of HF emphasizes both the evolution and progression of the disease (Heidenreich et al., 2022).

Stage of HF	Definition
A	High risk for developing HF with no evidence of cardiac structural or biomarker abnormalities
B	Cardiac structural or biomarker abnormalities but has never developed symptoms of HF
C	Current or previous symptoms of HF associated with underlying structural heart disease
D	End-stage disease who requires specialized treatment strategies

**Table 1. 1:** Classification of heart failure  
HF: Heart failure

### **1.2 Mechanism of ATRCD and concept of subclinical ATRCD**

Anthracycline inhibits topoisomerase II (Top2), an essential enzyme for unwinding deoxyribonucleic acid strands during deoxyribonucleic acid replication or transcription (Vejpongsa and Yeh, 2014). High cumulative dose of anthracycline expresses noxious effects on a wide range of cardiac tissues, including cardio myocytes, endothelial cells, fibroblasts, and cardiac stem cells (Yeh and Vejpongsa, 2015). In the cardiac tissue, anthracycline targets Top2 $\beta$ , the primary Top2 isoform in the heart, triggering profound changes in the transcription leading to defective mitochondrial biogenesis and reduced levels of anti-oxidative enzymes, manifested as increased production of reactive oxygen species and cardio myocyte death (Zhang et al., 2012). Anthracycline has also been shown to reduce coronary branching, capillary density, and the expression of myocardial vascular growth factors (Huang et al., 2010). The number of cardiac progenitor cells and their ability to differentiate into endothelial cells, smooth muscle cells, or myocytes is also diminished (Huang et al., 2010). Thus, the ability of the heart to adapt to any additional stress is impaired after exposure to anthracycline (Yeh and Vejpongsa, 2015).



**Figure 1. 1:** Mechanism of Anthracycline cardiotoxicity

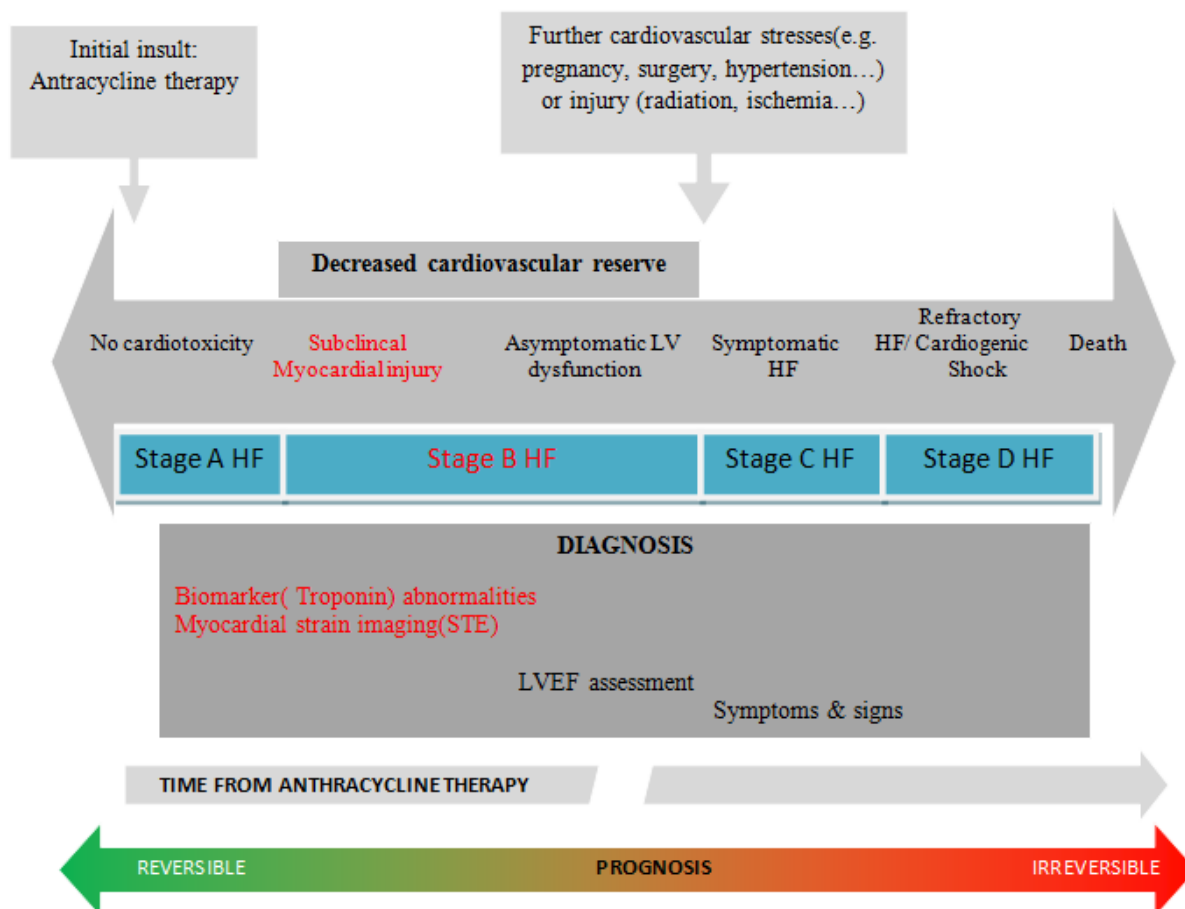
A: In the cardiac tissue, anthracycline inhibits topoisomerase II  $\beta$  (Top2 $\beta$ ), triggering profound changes in the transcription leading to defective mitochondrial biogenesis, increased production of reactive oxygen species and cardio myocyte death.

B: Anthracyclines induces deleterious effects on cardio myocytes, endothelial cells, fibroblasts, and cardiac progenitor cells, affects cardiac contractility, and attenuates repair, neovascularization and proliferation after injury, thus, results in cardiac dysfunction.

Findings from studies proposed that anthracycline cardiotoxicity represents a spectrum of cardiac tissue damage which starts with subclinical myocardial cell injury, followed by an early asymptomatic LVEF impairment which may worsen to clinical HF, if left without any intervention (Cardinale D, 2015). Patients with subclinical LV dysfunctions (stage B HF) may not all progress to stage C or D heart failure. However, these initial insults enhance cardiac susceptibility to further cardiovascular stresses (e.g. pregnancy, surgery, hypertension...) or injuries (radiation, ischemia...) and, ultimately, increase the risk of premature cardiovascular mortality. This phenomenon has been labeled as “multiple-hit” hypothesis (Lee et al., 1980).

Cardinale and colleagues suggested that the so-called “late-onset anthracycline cardiotoxicity” may just be “late” by the timing of diagnosis, rather than the timing of the onset of cardiotoxicity (Cardinale D, 2015) . These findings, together with the “multiple-hit” hypothesis, highlight an urgent need for the surveillance and management of anthracycline cardiotoxicity.

Periodic echocardiographic monitoring has been advocated for this vulnerable population (Lyon et al., 2022). To further improve the sensitivity of early detection of subclinical LV systolic dysfunction, cardio-oncology guidelines recommend to use global longitudinal strain (GLS) measured by speckle tracking ECHO (STE) imaging, often in conjunction with the measurement of blood levels of cardiac tissue injury biomarkers such as cardiac troponin (Thavendiranathan et al., 2014). It is thus advised to evaluate, at baseline (initiation of anthracycline regimen), LVEF, GLS, and circulating cardiac troponin. If any of these three parameters are abnormal, a cardiology consultation is recommended (Lyon et al., 2022). These are described in more details in section 1.5.



**Figure 1. 2:** Spectrum of ATRCD & "multiple - hit" hypothesis

HF: Heart Failure; LV: Left Ventricle; LVEF: Left Ventricular Ejection Fraction;  
STE: Spackle Tracking Echocardiography

### 1.3 Risk factors of ATRCD

It is always important to know who is at risk for developing ATRCD. There are a number of risk factors have been reported in the literature. They can be divided into patients-related and therapy- related risk factors. (Table 1.2)

#### 1.3.1 Patient-related risk factors

Patient-related risk factors include those with pre-existing or modifiable cardiovascular risk factors such as hypertension, diabetes mellitus, obesity, coronary artery disease (Albini et al.,

2010). Other non-modifiable risk factors are increasing age, gender and black race (Blanco et al., 2012).

### **1.3.1.1 Pre-existing cardiovascular risk factors**

Pre-existing cardiovascular risk factors have been suggested to be associated with increased incidences of ATRCD. It was reported that the incidence of cardiotoxicity in patients with hypertension, diabetes mellitus, or obesity is remarkably higher than that in normal patients (Cai et al., 2019). In a large-scale meta-analysis, Shuo Qiu and colleagues investigated the overall incidence of cardiotoxicity in cancer patients who were treated with anthracycline based chemotherapy and evaluated the associations between potential risk factors and cardiotoxicity. “Among the survivors of cancer chemotherapy, hypertension (OR: 1.99; 95% CI: 1.43–2.76), diabetes mellitus (OR: 1.74; 95% CI: 1.11–2.74), and obesity (OR: 1.72; 95% CI: 1.13–2.61) were revealed to be risk factors for ATRCD (Qiu et al., 2021)”. Among these, raised blood pressure is the most common cardiovascular risk factors presented in adult cancer patient (Blanco et al., 2012). In researches of anthracycline treated childhood cancer survivors, patients who developed hypertension in their adulthood, had significantly increases the risk of heart failure (Gregory T. Armstrong et al., 2013).

Pre-existing coronary heart disease, including myocardial infarction, previous coronary revascularization and stable angina, have been identified as risk factors of developing ATRCD (Lyon et al., 2020). In a large breast cancer cohort who received anthracycline based adjuvant chemotherapy, Pinder et al studied 43,338 women aged 66 to 80 years with stage I to III breast cancer. Coronary artery disease (HR: 1.58 95% CI: 1.39 to 1.79) was reported as highly significant predictor of a subsequent diagnosis of heart failure (Pinder et al., 2007).

Other conventional cardiovascular risks, such as smoking and menopause status have also been studied. However their link to ATRCD have not yet been well established (Reinbolt et al., 2016, Qiu et al., 2021).

The pre-existing cardiovascular risk factors in Uganda cancer patients who receive cardio toxic chemotherapy drugs like anthracycline have not been studied, nor have their predicting roles in ATRCD.

### **1.3.1.2 Ethnicity, gender and family history related risk**

Gender carries different risk of heart failure in general population (non-cancer population), with men often develop heart failure at a younger age than women. Women more commonly have heart failure with preserved ejection fraction (Heidenreich et al., 2022).

Studies investigating sexual dimorphism of anthracycline cardiotoxicity are still sparse. Yet, some experimental studies started showing more and more evidences that anthracycline cardiotoxicity may exhibit a sexual dimorphism with females being protected compared to males (Renée Ventura-Clapier et al., 2016). This protection includes the essential targets of anthracycline i.e. energy metabolism, energetic signaling pathways and oxidative stress (Renée Ventura-Clapier et al., 2016).

In contrast, when Gregory et al. reviewed studies of anthracycline cardiotoxicity in childhood cancer survivors, after identifying the 17 studies assessing sex as a risk factor for anthracycline cardiotoxicity, five studies, including the four high-quality studies, were found to show that females experience a poorer outcome than males (Armstrong et al., 2007). Gregory et al. further discussed the controversy on gender risk of anthracycline cardiotoxicity, that the incidence of anthracycline cardiotoxicity is higher in pre pubertal girls, and this could be explained by the

lacking protections from female hormone (Armstrong et al., 2007). Further studies are needed to understand in more details the mechanistic background of female protection.

The potential link between family history of cardiomyopathy and ATRCD has been proposed by case reports (van den Berg et al., 2010, Shipman and Arnold, 2011, Young et al., 2011). Wasielewski et al. carried out the first systematically investigation to prove the concept that a genetic/familial predisposition for dilated cardiomyopathy might be a potential risk factor for ATRCD (Wasielewski et al., 2014). They identified five cardiomyopathy families with one patient with ATRCD, and one patient with ATRCD with a family member with a possible early sign of mild cardiomyopathy. Pathogenic MYH7 mutations, the genetic markers of cardiomyopathy, were found in two of these families. However, the study is limited by the descriptive design and power. More robust research is needed to further investigate the association of familiar cardiomyopathy and ATRCD.

Black race was found to be a risk factor for developing ATRCD in both childhood cancer survivors (Krischer et al., 1997) and adult cancer patient (Hasan et al., 2004). This is described with more details in section 1.7.

### **1.3.2 Therapy-related risk factors**

Other risk factors of ATRCD are therapy-related, including use of combination cancer therapy, addition of mediastinal irradiation, and higher doses. Certain agents, such as trastuzumab, and cyclophosphamide, carry higher risk while others, such as bevacizumab, etoposide, and lapatinib, carry lower risk (Curigliano et al., 2010).

In a study investigating childhood cancer survivor, “after adjusting for age at diagnosis of cancer, sex, and chest radiation, there was a clear dose-dependent association between anthracycline exposure and cardiomyopathy risk. Compared with patients without anthracycline exposure, the

risk of cardiomyopathy increased with each dose category (1 to 100 mg/m<sup>2</sup>: OR, 1.65; 101 to 150 mg/m<sup>2</sup>: OR, 3.85; 151 to 200 mg/m<sup>2</sup>: OR, 3.69; 201 to 250 mg/m<sup>2</sup>: OR, 7.23; 251 to 300 mg/m<sup>2</sup>: OR, 23.47; and 300 mg/m<sup>2</sup>: OR, 27.59). Chest radiation was associated with an increase in risk of cardiomyopathy (OR, 4.29; 95% CI, 1.9 to 9.6; P <0 .001) (Blanco et al., 2012).”

<b>Patient-related risk factors</b>
Hypertension
Diabetes mellitus
Obesity
Previous heart disease
Old age
? Female
? Family history of cardiomyopathy
Black race
<b>Therapy-related risk factors</b>
Higher anthracycline dose
Combination therapy
Mediastinal irradiation

**Table 1. 2:** Risk factors for anthracycline therapy related cardiac dysfunction

#### **1.4 Epidemiology and nature history of ATRCD**

In the literature, there is wide variation in the reported frequency of both clinical and subclinical cardiotoxicities. In children, early onset cardiotoxicity seems to occur less frequently than late cardiac damage. There is a relatively low rate of early clinical cardiotoxicity, since only 1.6% of all children treated with anthracycline had cardio toxic effects (Krischer et al., 1997). In a systemic review conducted by Kremer and colleagues, the incidence of late onset ATRCD in childhood cancer survivors was estimated in 30 studies described in 25 articles. The frequency varied between 0% and 16% (Kremer et al., 2002). Among these 30 studies, the highest ATRCD incidence was reported by Gilladoga’s group, who investigated 50 children, ranging in age from 8 months to 15 years when doxorubicin was first administered, reached cumulative doses exceeding 500mg/m<sup>2</sup> between September,1971 and August,1974. These children were observed for periods

of 11-57 month. Eight children, in addition, received mediastinum radiation. Of these 50 children, 8 (16%) developed severe cardiomyopathy with congestive heart failure (Gilladoga et al., 1976). Cardinale and colleagues assessed LVEF, “at baseline, every 3 months during chemotherapy and for the following year, every 6 months over the following 4 years, and yearly afterward in a heterogeneous cohort of 2625 adult patients receiving anthracycline-containing therapy. In case of cardiotoxicity (LVEF decrease >10 absolute points, and <50%), HF therapy was initiated. Recovery from cardiotoxicity was defined as partial (LVEF increase >5 absolute points and >50%) or full (LVEF increase to the baseline value). The median follow-up was 5.2 (quartile 1 to quartile 3, 2.6–8.0) years. The overall incidence of cardiotoxicity was 9% (n=226). The median time elapsed between the end of chemotherapy and cardiotoxicity development was 3.5 (quartile 1 to quartile 3, 3–6) months. In 98% of cases (n=221), cardiotoxicity occurred within the first year. Twenty-five (11%) patients had full recovery, and 160 (71%) patients had partial recovery (Cardinale D, 2015)”. The cumulative incidence of ATRCD (22%) was high in a small Uganda cancer study (Kibudde et al., 2019). This wide variability could be due to the differences in study population, treatment protocols, and duration of follow-up.

Only few studies reported the incidence of subclinical ATRCD. In a cohort of 140 breast cancer patients who received anthracycline therapy, Boyd, A. group reported an incidence of 22% of subclinical LV dysfunction (defined by >11% reduction in GLS) using 2D strain analysis within 3 months after anthracycline chemotherapy (Boyd et al., 2017). In another cohort, Negishi et al followed up 159 patients receiving anthracycline, trastuzumab (a monoclonal antibody for treating HER2 receptor positive breast cancer), or both (Negishi et al., 2014). They found 33% of the patients developed decreased GLS (by >11%) in 7 months after the completion of the chemotherapy. It is need to be noted that in both studies, LVEF remained within normal ranges.

## 1.5 Diagnosis

### 1.5.1 Detection of LV Dysfunction Using Two-Dimensional Echocardiography imaging

Exposure to potentially cardio toxic chemotherapeutic agents is a well-recognized indication for baseline and longitudinal evaluation of LV function (Daher et al., 2011, Douglas et al., 2011).

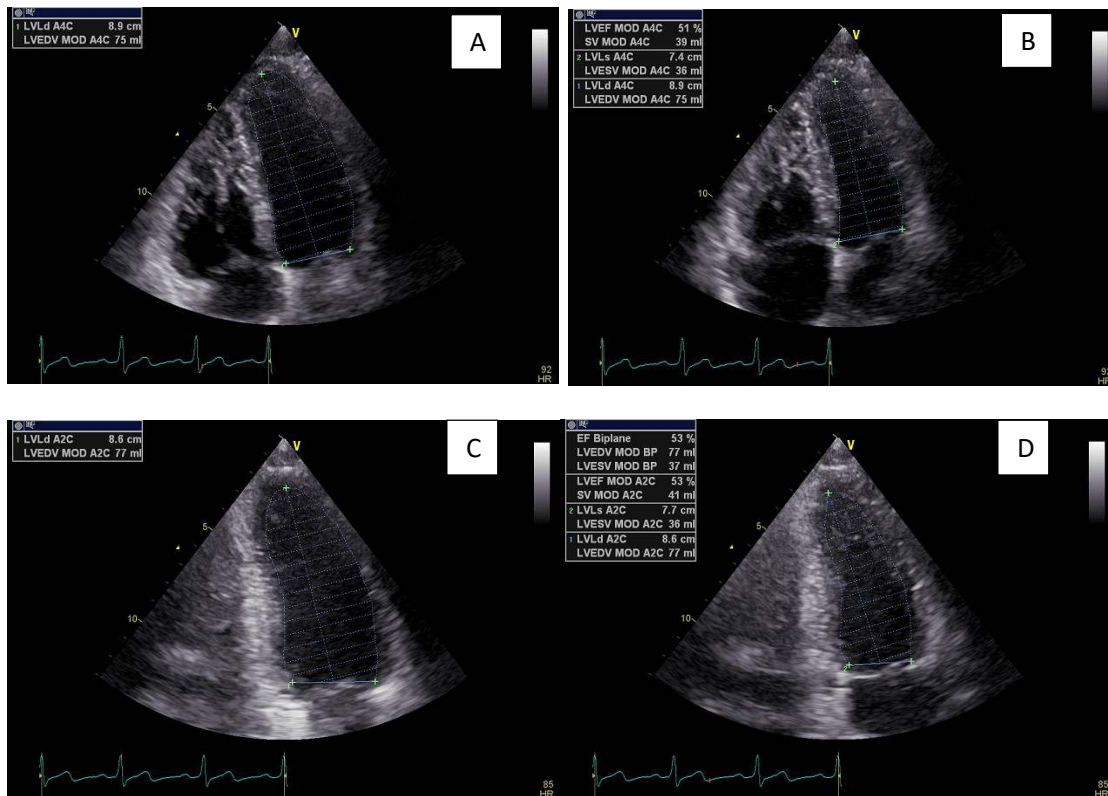
“Because of its widespread availability and safety, 2D ECHO is increasingly used in monitoring patients with cancer. 2D ECHO allows for characterization of systolic and diastolic function, pulmonary pressures, valvular function, right ventricular (RV) function, and the pericardium. The most commonly used parameter for monitoring LV function with ECHO is LVEF (Oh JK, 2006).”

LVEF is measured using volumetric measurements as described by the following equation:

$$\text{LVEF} = (\text{LVEDV} - \text{LVESV}) / \text{LVEDV}$$

where LVEDV and LVESV are LV end-diastolic volume and end-systolic volume, respectively.

According to joint recommendations from the American Society of Echocardiography (ASE), and the European Association of Echocardiography (EAE), the method of choice for LV volumes quantitation and LVEF calculation is the modified biplane Simpson’s technique (method of disks) by 2D ECHO (**Figures 1.3**) (Lang et al., 2015). The recommendations for chamber quantification from the ASE and EAE established  $\text{LVEF} \geq 50\%$  as a normal reference range (Lang et al., 2015). ATRCD is defined as a decrease in the LVEF of  $>10$  percentage points, to a value  $<50\%$  (Plana et al., 2014).



**Figure 1. 3:** Calculation of LVEF using the biplane Simpson’s method

(A) Apical four-chamber view obtained at end-diastole. (B) Apical four-chamber view obtained at end-systole. (C) Apical two-chamber view obtained at end-diastole. (D) Apical two-chamber view obtained at end-systole.

“Assessment of LVEF is based on assumptions of cardiac geometry, depends on image quality, cannot detect small regional alterations in myocardial function, and may vary based on loading conditions(Cheitlin et al., 2003).” One of the limitations of 2D ECHO is that it almost unable to detect small (<10%) changes in LVEF (Plana et al., 2014). However, for cancer patients, a subtle difference in cardiac function can be so vital to guide on treatment dose adjustment or cessation.

### 1.5.2 Detection of Subclinical LV Dysfunction Using Strain and Speckle Tracking Imaging

More recent techniques, including strain with STE, may allow for earlier detection of more subtle changes in myocardial function (Plana et al., 2014). Strain is defined as the change in length of an object relative to its original length. In the heart, myocardial strain is a sensitive measure of

contractility. Strain can be calculated at both the segmental and global level and in the three axes of myocardial contraction - circumferential, longitudinal and radial (**Figure 1.4**).



**Figure 1. 4:** Myocardial contraction in three vectors- circumferential, longitudinal and radial.

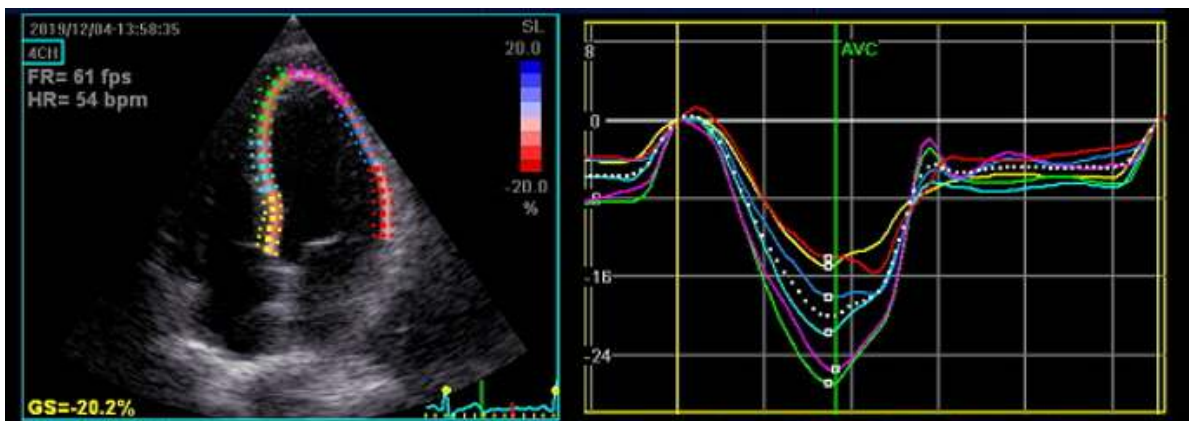
LV: Left ventricle

Tissue Doppler imaging can assess myocardial strain but this technique is extremely angle dependent and has been superseded by STE (Feigenbaum et al., 2012, Gorcsan and Tanaka, 2011). The ultrasonic images obtained by ECHO consist of a large number of “speckles” which have individual properties (Chan). These “acoustic markers” can be identified and tracked as they move from one frame to the other throughout the cardiac cycle (Gorcsan and Tanaka, 2011). Endocardial and pericardial borders are pre-defined by the operator and each speckle within this region of interest (ROI) is tracked. The tracking of such movement can be used to derive measures of strain (Flachskampf et al., 2011).

Most laboratories document LV strain in the long axis and make use of the GLS calculated as the mean from each segment, as a measure of global LV function. **Figure 1.5** indicates characteristic segmental strain traces in a normal heart. “Images for GLS are made in standard apical two-, three-, and four-chamber views and aortic valve closure (AVC) is used for timing of end-systole (Lang et al., 2015).” It is reported that the normal GLS for a healthy heart ranges from -18% to -25%, which information is accepted by most of the echocardiographic laboratories. However,

intersoftware and intervendedor variability might contribute to some degrees of variations in GLS measurement. Short axis views allow circumferential and radial strain to be derived. But, it is difficult to accurately obtain global measures due to uncertainty of the imaging plane location.

In the setting of cancer patients, GLS is proved to be promising in early detection of chemotherapy related cardiac dysfunction. GLS reduction precedes LV dysfunction in patients who later develop HF. Strain decline can be manifested earlier while LVEF is preserved (Oikonomou et al., 2019, Lambert et al., 2020). Persistent strain abnormalities were observed in patients receiving high-dose anthracycline (Stoodley et al., 2013). In patients receiving trastuzumab alone or with anthracyclines, a change in GLS of  $>11\%$  was the strongest predictor of cardiotoxicity (Negishi et al., 2013). The drawbacks of this techniques are limited availability, image quality dependent, variability of quantification between vendors, and lack of universal definitions (Plana et al., 2014).



**Figure 1. 5:** Segmental strains in apical four-chamber view, showing normal contractions.

The color of each trace corresponds to anatomical points on the 2-D color image to the left. The white dotted line represents average strain.

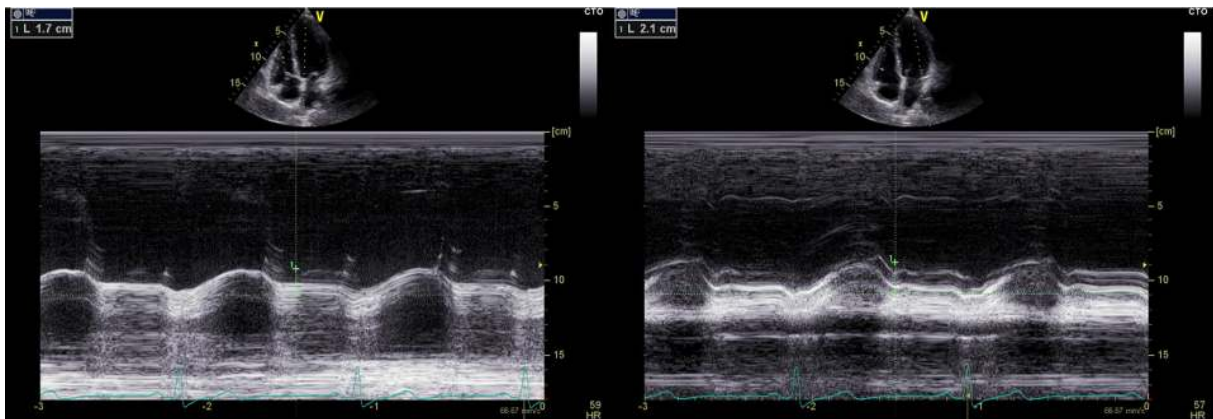
### 1.5.3 Detection of Subclinical LV Dysfunction using conventional ECHO parameters

When STE technology is not available, the quantitation of LV longitudinal function by simple ultrasound tools such as mitral annular plane systolic excursion (MAPSE) or mitral annular peak systolic velocity ( $S'$ ) could be useful adjunct information to LVEF in the evaluation of LV systolic

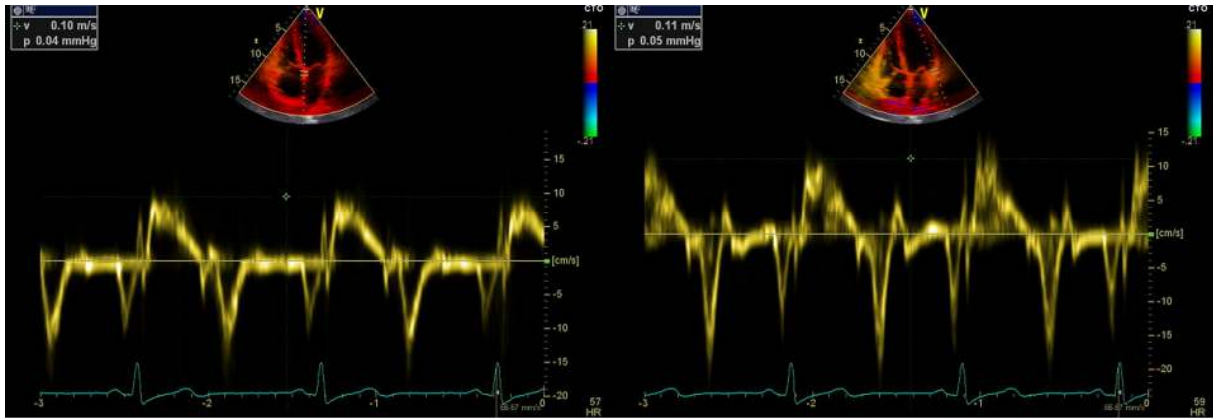
function (Ewer MS and SM, 2009, Karakurt et al., 2008, Ganame et al., 2007, Tassan-Mangina et al., 2006, Kapusta et al., 2000).

MAPSE is the horizontal distance between mitral annular end diastolic and peak systolic position. It is measured by M mode imaging from the apical 4 chamber view. Septal MAPSE can be obtained by placing the cursor across the septal mitral annulus, while lateral MAPSE is obtained when the cursor is placed across the later mitral annulus (**Figure 1.6**). S' represents the mitral annular peak systolic velocity, measured by pulse wave Tissue Doppler image (TDI) at the apical 4 chamber view (Figure 1.7). Septal and lateral S' are measured when the pulse wave TDI is sampled at the septal and lateral mitral annulus respectively (**Figure 1.7**).

MAPSE and S' are less dependent on image quality. However, no study has defined the cutoff values that allow the prediction of ATRCD. The current international recommendation is that a progressive decline of MAPSE should raise concern for subclinical LV dysfunction (Plana et al., 2014).



**Figure 1. 6:** Septal and lateral mitral annular plane systolic excursion (MAPSE) measured by M mode echocardiography



**Figure 1. 7:** Septal and lateral peak systolic velocity ( $S'$ ) of the mitral annulus measured by pulsed-wave tissue Doppler

### 1.5.4 Detection of Subclinical LV Dysfunction Using Biomarkers

A biomarker approach for early identification, risk stratification and monitoring of ATRCD holds promise, although challenges exist with respect to timing of measurement, optimal assays, and whether this strategy is best used alone or in conjunction with imaging.

#### 1.5.4.1 Biomarker of Injury (E.g. Troponin)

Troponin T (TnT) and Troponin I (TnI) are cardiac regulatory proteins that control the calcium mediated interaction between actin and myosin. The cardiac forms of these regulatory proteins are coded by specific genes and theoretically have the potential of being unique to the myocardium. Indeed, TnI has not been identified outside the myocardium (Bodor et al., 1995). TnT is expressed to a small extent in skeletal muscle. However, the current TnT assay does not identify skeletal troponin (Apple et al., 1998). Troponin is released when the cardiac muscle has been damaged, such as occurs with acute ischemia, toxins... The measurement of serum TnI and TnT is superior in terms of sensitivity and specificity to cardiac muscle enzyme measurements in the identification of cardiac muscle damage (Collinson et al., 2001).

“Troponin is probably the best characterized marker for evaluating chemotherapy induced cardiac injury (Christenson et al., 2015).” Currently, most of the studies have looked at anthracycline based therapy with limited clinical application. Despite these reservations, it was concluded, by an expert committee report to the FDA in the year 2004, that TnI and TnT are in fact sensitive, specific, and strong markers of cardiac cell injury, this allowing for the identification and quantification cellular injury, as well as death, associated with the testing of new drugs (Wallace et al., 2004).

Measurement of circulating troponin level pre and post every anthracycline therapy cycle can be useful for the early detection of the cardiac dysfunction. In patients who received high doses of anthracycline without evidence cardiac dysfunction, the absence of troponin elevation indicated a high negative predictive value of this biomarker (Cardinale et al., 2004). Sawaya et al. also conclude that “ TnI levels at completion of anthracycline treatment were predictive of subsequent reduction in LVEF and cardiac events (Sawaya et al., 2012).”

#### **1.5.4.2 Biomarker of Load (E.g. Natriuretic Peptide)**

Natriuretic peptides, such as brain-type natriuretic peptide (BNP) and N-terminal pro-BNP (NT-proBNP), are small peptides synthesized and released from the atria and ventricles when the heart is stretched in the condition of overloading (D'Alessandro et al., 2013). The release of these peptides can lead to natriuresis, vasodilation, and diuresis through the pathway mediated by cyclic guanosine monophosphate (cGMP) (D'Alessandro et al., 2013). Natriuretic peptides have been accepted historically and are widely used biomarkers in heart failure diagnosis, treatment monitoring, and risk stratification (Plana et al., 2014).

As one of the most reliable biomarkers of heart disease, natriuretic peptides certainly cannot be missed out by ATRCD investigators. In one of the trials, Romano et al. found out that BNP was

able to predict LV dysfunction at different follow up intervals (Romano et al., 2011). A rise in natriuretic peptides during the first 90 days was predictive of LV dysfunction at 4 years among anthracycline treated individuals (Lipshultz et al., 2012b). Nevertheless, natriuretic peptides has failed, in some studies, to display any association with cardiac toxicity resulting from anthracycline therapy (Sawaya et al., 2012, Onitilo et al., 2012). Regardless, their negative predicted value might be more reliable for the cardiac monitoring strategy (Bloom et al., 2016). Continuous studies are required for the exploration of the utility of natriuretic peptide employment in anthracycline treated patients.

#### **1.5.4.3 Other Biomarkers**

High-sensitivity C reactive protein (hs-CRP) is one of the nonspecific cardiac biomarkers which has been evaluated as a potential predictor for anthracycline cardiotoxicity. But the heterogeneous results are largely inconclusive. In a study, “left ventricular dysfunction was noted to correlate with hs-CRP levels in childhood cancer survivors not exposed to cardio toxic therapy suggesting that the hs-CRP may be a surrogate for overall inflammation or tumor burden in addition to drug effects (Lipshultz et al., 2012a).” Other studies of patients receiving anthracycline-containing regimen followed by taxanes and trastuzumab showed no association between C-reactive protein, galectin-3, ST-2 or growth differentiation factor (Cardinale D et al., 2010) and cardiotoxicity (Cardinale D et al., 2010).

Myeloperoxidase (MPO) is an enzyme released by neutrophils. It is proatherogenic in nature and promotes free radical production and lipid peroxidation (Ky et al., 2014). The result of Ky et al. study reported MPO levels after anthracycline administration were associated with the later development of cardiotoxicity (Ky et al., 2014). Another study of multiple biomarkers found MPO levels not only “rose early, persisted throughout the course of therapy, but also were associated

with cardiotoxicity (Putt et al., 2015).” However more robust studies with longer follow-up period are needed to verified the utility of MPO as a predictor of anthracycline cardiotoxicity.

### **1.5.5 Detection of Subclinical LV Dysfunction – An Integrated Approach of Imaging and Biomarkers**

“An integrated approach combining echocardiographic data and biomarkers may be useful and provide incremental value in predicting subsequent ATRCD. It may also provide a strategy for more aggressive surveillance if used in parallel or reduction in the frequency of imaging when used in series (i.e., alternating imaging with biomarkers) (Plana et al., 2014).” A study done in the anthracycline and trastuzumab treated breast cancer patients by Sawaya et al., showed that the monitoring of ultrasensitive troponin levels and STE-derived GLS at the same time after anthracycline therapy has improved specificity of 93%, in comparison with either parameter alone (73%) (Sawaya et al., 2012) .

### **1.5.6 Current recommendation by ASE**

In order to improve the cardiovascular care in cancer patients, standardize the cardio-oncology practice, ASE in conjunction with European association of cardiovascular imaging, launched guidelines for imaging evaluation of adult patients during and after cancer therapy based on expert consensus (Plana et al., 2014).

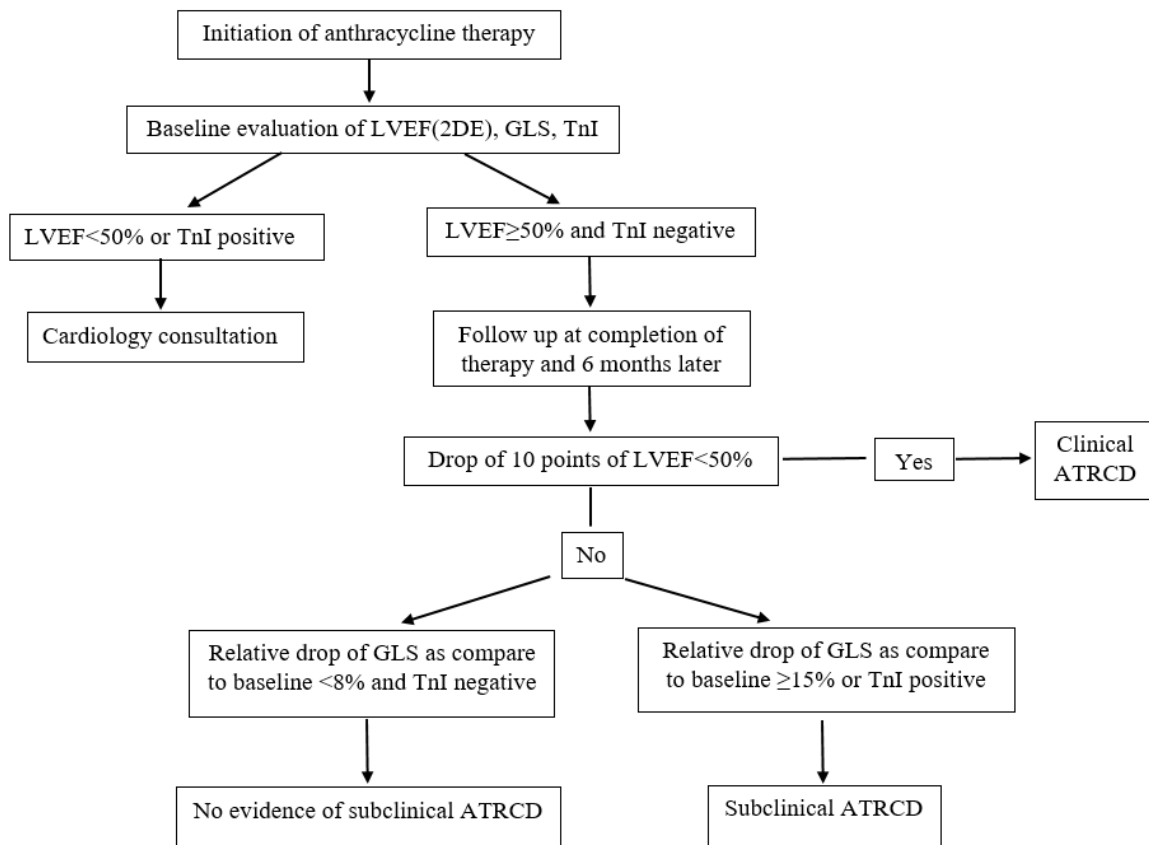
#### **1.5.6.1 Cardiac assessment and monitoring protocol (Figure 1.8)**

“At the initiation of anthracycline, a baseline evaluation including measurements of LVEF, GLS, and troponin is recommended. If any are abnormal, a cardiology consultation is recommended. Follow-up is recommended at the completion of therapy and 6 months later for doses < 240 mg/m<sup>2</sup>

or its equivalent. Once this dose is exceeded, measurements of LVEF, GLS, and troponin are recommended before each additional 50 mg/m<sup>2</sup> (Plana et al., 2014)".

### 1.5.6.2 Early detection of subclinical LV dysfunction

In the absence of adjudication of ATRCD, it is recommended to use GLS and TnI for the identification of subclinical LV dysfunction. If baseline strain is available, a relative percentage decrease of GLS  $\geq 15\%$ , compared with baseline and /or TnI become positive during follow up is likely to be of clinical significance.



**Figure 1. 8:** Cardiac monitoring protocol of patient who receives anthracycline therapy

## **1.6 Treatment of subclinical LV dysfunction**

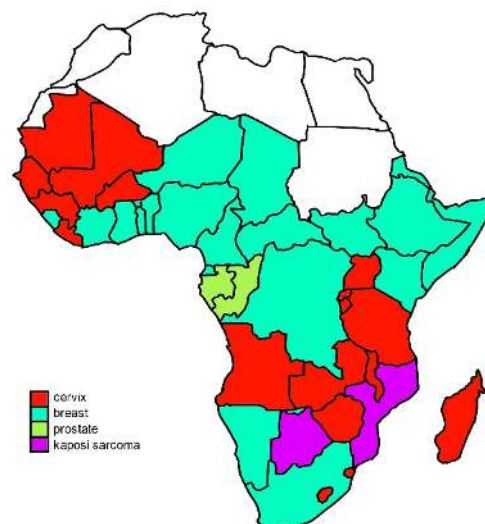
Although combination regimens for HF therapy have been reported to be effective, HF due to ATRCD is often resistant to therapy if diagnosed late in its course (Plana et al., 2014). Hence, prevention of HF should be the primary goal of the effort. The reasonable approach to HF prevention is to detect early and treat early. “To appreciation of the implication of against subclinical LV dysfunction prevention is a key step in developing a screening strategy. There would be no purpose in screening if there were no therapeutic implications (Plana et al., 2014).” Currently, data support initiating cardioprotective drugs for the treatment of subclinical ATRCD is limited. The most effective agents for primary prevention appear to be dexrazoxane (Marty et al., 2006). Dexrazoxane is a strong iron chelator that has the ability to displace iron from the anthracycline (Hasinoff BB, 1995). Dexrazoxane is also a catalytic inhibitor of DNA Top II that is the same target as the anthracycline (Lyu et al., 2007). However, concerns have been raised right from the mechanism of which dexrazoxane exhibits its cardioprotective effect, that this drug may attenuate anthracycline anti-tumor activity (Lyu et al., 2007).

More and more evidences suggest beta-blockers are cardioprotective and have a role on prevention of anthracycline-induced cardiotoxicity. Carvedilol has attracted a lot of attention, because of its antioxidant and iron chelator properties. In an experimental study, carvedilol was found to prevented cardiac histopathology caused by anthracycline (Oliveira et al., 2004). Carvedilol has as well showed effective to prevent strain decline after anthracycline exposure (Elitok A et al., 2014). In studies with carvedilol (Kalay et al., 2006) and nebivolol (Kaya et al., 2013) started at initiation of anthracycline treatment, both drugs resulted in lower degree of LVEF decline. Seicean et al. also reported a lower incidence of HF over a 5-year period with the concomitant use of beta-blocker during treatment with trastuzumab and anthracyclines (Seicean et al., 2013). While

carvedilol and nebivolol are beneficial, nonselective beta blockers such as propranolol may be instead cardio toxic (Choe et al., 1978) and the effect of metoprolol is neutral (Georgakopoulos et al., 2010).

### 1.7 African population who are at risk of developing ATRCD

Social and economic transition in Sub-Saharan Africa (SSA) has brought population aging and growth, followed by a paradigm shift of disease epidemiology from communicable disease to non-communicable disease including cancer. Cancer is emerging as a major public health problem in SSA driven by increased prevalence of traditional risk factors, as well as a high residual burden of infectious agents (HIV/AIDS, human papillomavirus, hepatitis B virus). It is estimated that about one-third of all cancers in the region are related to infection (Atlas., 2022). “Breast and cervical cancer in women and prostate cancer in men are the major cancers happening with bad outcome in SSA (Atlas., 2022).” (Figure 1.9)



**Figure 1. 9:** The most common cancer cases in sub-Saharan Africa, both sexes combined, 2018

Today, anthracycline is playing an important role in cancer management, because of the growing burden and the unique pattern of cancer in SSA. In high income countries, the use of anthracycline has been gradually reduced with the emerging of novel, less cardiac toxic antitumor drugs when treating certain types of cancer (Spallarossa et al., 2016). However, the high cost of the novel regimen has largely limited its utility in SSA countries. Following the launching of the African Cancer Network Project in 2012, more than a hundred cancer treatment institutions have been set up by 2015 (Stefan, 2015). Cancer patients in Africa are having better access to anthracycline based chemotherapy. While true data reporting the number of patients who receive or need anthracycline is lacking in the continent, “it has been estimated that about 60% of cancer patients in Uganda Cancer Institute (UCI) are treated with anthracycline (Kibudde et al., 2019).” The common cancers treated with anthracyclines at UCI, include breast cancer (70.6%), Non-Hodgkin’s lymphoma (9.6%), Hodgkin’s Lymphoma (7.3%), advanced hepatocellular cancer (1.1%), soft tissue sarcomas (8.2%) and leukemia (2.0%). It should be noted that, women accounted for 80% of this population who is at risk of cardiotoxicity in UCI (Zhang et al., 2021).

Studies in childhood cancer survivors (Krischer et al., 1997) and adult cancer population (Hasan et al., 2004, Pinder et al., 2007) suggest black ethnicity may hold higher risk for developing anthracycline cardiotoxicity. Furthermore, Rong Stephanie Huang used EBV-transformed B-lymphoblastoid HapMap cell lines derived from an African descent and a European descent cell line, in order to evaluate population- and gender specific differences in cell cardiotoxicity after daunorubicin, and other drugs (carboplatin, cisplatin, etoposide) treatment. Interestingly, African descent cell lines were found to be more prone to develop cyto-toxicity linked to daunorubicin (Huang et al., 2007).

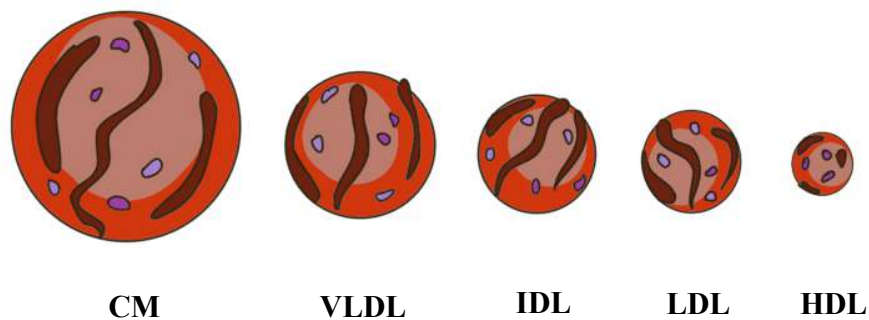
In Africa, two published studies done in Cote d'Ivoire and Morocco, reported high incidence of cardio toxicity in adult cancer patients on anthracycline treatment. Elalouani et al. who conducted the first prospective cohort study in Morocco investigating the frequency of anthracycline induced cardiotoxicity noted that 56% of the 70 patients developed a decrease in cardiac function and 4% of cases developed severe cardiotoxicity (Elalouani et al., 2012). In the prospective cohort study performed at Abidjan institute of cardiology over 10 months, 45 adult patients were followed up and 4 patients (8.8%) developed significant cardiotoxicity (Pio et al., 2013).

### **1.8 Anthracycline 's other effect on cardiovascular health- lipid homeostasis**

Anthracycline therapy has significant impact on patient's cardiovascular health through different ways, with ATRCD is the most common and well known for the last five decades. Today, findings from the researches investigating how anthracycline alters patients' cardiovascular risks brought increasing attention on its actions on lipid homeostasis.

#### **1.8.1 Lipoprotein subtractions and cardiovascular risk**

There are three types of lipid in the serum: triglycerides, cholesterol and fatty acids. In order to be transported all over the body, most of the triglycerides and cholesterol need to be bonded with proteins called apolipoproteins to form lipoproteins (Sharadendu Bali, 2019). Lipoproteins are classified into five types, according to their size and density. These are, from the largest to smallest size, chylomicrons (CM), very low density lipoprotein (VLDL), intermediate density lipoproteins (IDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) (**Figure 1.10**). The lipoproteins can also be broadly divided into two groups according to the main apolipoprotein they contain: ApoB lipoprotein (also called non-HLD, including VLDL, IDL and LDL) and ApoA lipoprotein (HDL) (Sharadendu Bali, 2019).



**Figure 1. 10:** Lipoprotein types

CM: chylomicrons; VLDL: very low density lipoprotein; IDL: intermediate density lipoproteins; LDL: low density lipoprotein; HDL: high density lipoprotein

Traditionally, high LDL or low HDL are the major risk factors of cardiovascular disease (Warnick and Wood, 1995, Bachorik and Ross, 1995). However, the role of lipoprotein concentration measurement in cardiovascular risk evaluation is limited among patients who develop cardiovascular disease with a “normal” lipoprotein level (Sachdeva et al., 2009).

It is known that the lipoprotein can be further sub classified into multiple subfractions that vary with respect to particle size, density and chemical composition. The value of lipoprotein subfraction levels as an aid in the assessment of cardiovascular risk has been investigated using different methods. Studies have reported that lipoprotein subfractions in general, and LDL in particular, are associated with cardiovascular events (Shiffman et al., 2017, Mora et al., 2015). Among LDL subfractions, small dense LDL is more atherogenic than larger LDL (Lamarche et al., 1997) and is associated with an increased risk for coronary artery disease (Rosenson et al., 2002). Two main subclass patterns of LDL have been suggested depended on predominant LDL particle size: large, buoyant LDL pattern A and small dense LDL pattern B (Austin and Krauss, 1986). LDL pattern B is independently associated with higher rate of cardiovascular disease

progression (Austin et al., 1988, St-Pierre et al., 2005). LDL pattern B prevalence in young adults has been reported to be 18.5–29.2% (Watson et al., 1994, Srinivasan et al., 2001). Malhotra and colleagues reported a prevalence of 36.4% of LDL pattern B in long term survivors of acute lymphoblastic leukemia (Malhotra et al., 2012)

### **1.8.2 Anthracycline alters lipid homeostasis**

Anthracycline has been shown to increase non HDL (VLDL, IDL and LDL) and decrease HDL, hence alter cardiovascular risks in cancer survivors (Giskeødegård et al., 2022, Dong et al., 2022, Xu et al., 2020, Lu et al., 2020). How anthracycline alters lipid homeostasis is not completely understood. Several mechanisms have been proposed: 1. Anthracycline may directly cause endothelial dysfunction and insulin resistance, leading to cytokine alterations, finally increasing serum lipid profiles (Kim et al., 2006, Finkelman et al., 2017, Sonowal et al., 2018). 2.

Anthracycline therapy related oxidative stress enhancement causes lipid peroxidation which is toxic to the liver, the vital lipid homeostasis regulating organ (Ma et al., 2002). 3. Anthracycline increases the expression of ApoB in liver cells, thus increase the production of ApoB lipoproteins (Sharma et al., 2016).

How dose anthracycline affect lipid subfractions, particularly LDL subfractions pattern, has never been studied.

### **1.9 Conclusion**

Anthracyclines are highly effective antineoplastic regimens which are commonly used globally in oncology practice, including Africa. A growing concern of ATRCD affecting the long term outcome of cancer survivors has promoted more researches and technologies that result in early detection, early management, therefore, better prevention and clinical outcomes. These technologies and strategies include detecting ATRCD at subclinical and asymptomatic stages

with LVEF by 2D ECHO, GLS by STE and cardiac TnI level in the blood. These modalities are to be performed on patients, who receive anthracycline therapy, at baseline and sub sequentially following the chemotherapy to achieve timely detection of ATRCD, appropriate intervention and prevention of severe heart failure.

Dyslipidemia is another anthracycline therapy related side effect being proposed by contemporary researchers. However, more studies are needed to confirm and understand anthracycline's effect on lipid homeostasis.

With the increasing burden of cancer and improved oncology care in Africa, more and more African cancer patients are receiving anthracycline based chemo-therapy, given its advantages of highly effective and relatively affordable. Behind the blessing of anthracycline to African cancer patients is a large population who is at the potential risk of ATRCD.

So, following above literature review, we would like to ask ourselves: What are the current status and challenges of cardiac care for African population who receives anthracycline based chemotherapy?

**N.B:** *The definition and classification of ATRCD have just been updated in August 2022 by European Society of Cardiology (ESC) and International Cardio-Oncology Society (ICOS) following the completion of this thesis. The updated definition and classification are not contradicting with the content of this assay. In order to not interact with the flow of this thesis, we present, in the Appendices section, the updated definition and classification of ATRCD and how the terms used in this thesis are correlate to the new terms.*

## **Chapter 2: Rational, Aims, Hypothesis and Objectives**

Despite an increased risk of developing ATRCD in Africa, there is a noted paucity of information on burden of subclinical anthracycline induced cardiotoxicity and related predictors among adult cancer patients receiving anthracycline chemotherapy. This big gap in knowledge has led to a lack of local guidelines for monitoring and management of ATRCD. This chapter presents the current cardiac care in Uganda cancer patients, identifies the challenges in this area and describes the rational and aims of the SATRCD study.

### **2.0 Background**

Anthracyclines are potent antineoplastic agents with proven efficacy in the treatment of many pediatric and adult hematologic and solid organ cancers. Dose-dependent anthracycline-induced cardiomyopathy is the most notorious and well-studied chemotherapy-induced cardiovascular toxicity that was first described in 1971 in 67 patients treated with Adriamycin for a variety of tumors (Middleman et al., 1971). The clinical significance of anthracycline cardiotoxicity is growing with increasing cancer survivorship worldwide (Strongman et al., 2019). Despite the wide use of anthracyclines, their cardio toxic mechanism is still not completely understood. The most common hypothesis is the formation of free radicals and super oxides, which lead to oxidative stress (Rossi F et al., 1994; Rajagopalan et al., 1988, Albin et al., 2010, Vasquez-Vivar et al., 1997). Cardiac cells are more susceptible to free radical damage. Findings from studies proposed that anthracycline cardiotoxicity represents a spectrum of cardiac tissue damage which starts with subclinical myocardial cell injury, followed by an early asymptomatic LVEF impairment which may worsen to clinical heart failure, if left without any intervention (Cardinale et al., 2015). The emerging concept of subclinical ATRCD implies the significance of the surveillance and management of anthracycline cardiotoxicity. A new ECHO technique, GLS by STE, together with biomarkers (TnI), have been recognized as the main diagnostic tools for detecting ATRCD as early

as subclinical stage. These modalities have been recommended by the current cardio-oncology guidelines (Lyon et al., 2022). When STE is not available or not feasible, simple ECHO parameters, which represent the left ventricular longitudinal systolic function, may serve as the surrogate of GLS in resource limited settings. However, their accuracy for screening subclinical ATRCD have not been assessed.

Cancer is emerging as a major public health problem in SSA because of population aging and growth (Guwatudde et al., 2015). Given the background of rising burden of cancer in SSA, the need of anthracycline in cancer treatment is also increasing in SSA (Stefan, 2015). African ethnicity was suggested having higher risk factor for developing ATRCD in the studies of adult cancer patients (Hasan et al., 2004, Pinder et al., 2007), as well as childhood cancer survivors (Krischer et al., 1997). Today, in SSA, well collected data is still lacking to address the epidemiology status of ATRCD, such as the incidence and predictors of the condition, particular the information of subclinical disease which is a silent risk factors for symptomatic HF. The cost implication of cardiac monitoring in addition to the knowledge gaps of the disease result in no locally established guidelines for the cardiac care of this vulnerable population in the continent.

There is no published data in Uganda regarding the burden and risk factor of clinical or subclinical ATRCD, though a relatively high incidence of ATRCD was described in a small study (Kibudde et al., 2019).

There is also growing concern that anthracycline therapy can increase cardiovascular risk in cancer survivors through its adverse effect on lipid homeostasis (He et al., 2020, Dong et al., 2022). This has not been investigated in Uganda patients as well.

## **2.1 State of the problem**

In Uganda, ATRCD is commonly recognized at the stage of symptomatic HF (stage C or D heart failure). Usually, patients do not respond well to heart failure medicines and have poor outcomes. More than half of the patients in UCI require anthracycline based chemotherapy, yet only baseline ECHO was performed as a routine on the majority patients. Without follow up cardiac assessment may be at a cost of leaving many patients go with a silent subclinical myocardial injury until the symptomatic HF developed with or without a triggered, after they have survived the cancer.

Our understanding of ATRCD comes mainly from the data of the high income countries. However, using international recommendation to detect subclinical ATRCD in Uganda, a low-income country, is challenging, due to limited availability of STE.

While the traditional anthracycline therapy related cardiotoxicity is still threatening the life in Ugandan patients, its side effects have been extended to alter lipid hemostasis which implies additional impact on cardiovascular health in anthracycline treated cancer survivors.

To date, there is no contemporary data in Uganda to address the burden and risk factors of subclinical ATRCD. How anthracycline affect the patients' lipid subfractions pattern is largely unknown as well. This big gap in knowledge has led to a lack of local guidelines for the cardiovascular care in anthracycline treated cancer survivors.

## **2.2 Study justification**

Detecting ATRCD at the subclinical stage is very important for the long term outcome of cancer survivors. Therefore, this study aims to fill the gaps in knowledge in Uganda cancer patients who are treated with anthracycline by studying the burden, risk factors and identifying other available conventional ECHO parameters that are able to detect subclinical ATRCD. This study further

investigates the lipid subfractions change following anthracycline therapy. Therefore, the results of the study will promote the application of available resources in cardio-oncology clinical practice, help to establish national guidelines on cardiovascular care of patients who receive anthracycline therapy.

### **2.3 Hypothesis**

We hypothesize that pre-existing cardiovascular risk factors predict the development of cardio toxicity in Ugandan adult cancer patients treated with anthracycline. Conventional ECHO parameters play important roles in detecting subclinical ATRCD in Ugandan adult cancer patients. Anthracycline therapy affects the LDL subfractions pattern, thus, alters patients cardiovascular risk.

#### **Hypothesis to be tested:**

- Whether the pre-existing cardiovascular risk factor(s) can predict(s) the development of ATRCD in Ugandan adult cancer patients?
- Whether conventional ECHO parameters (MAPSE, S'), have a correlation with GLS and are able to diagnose subclinical ATRCD at the end of the therapy?
- Whether anthracycline can change the LDL subfractions pattern in selected patients with low baseline cardiovascular risk at the end of the therapy?

### **2.4 Aim of the Study**

The aim of this research is to describe the burden of subclinical ATRCD in Ugandan cancer patients, identify the risk factors of developing anthracycline cardiotoxicity in the study population, evaluate the role of conventional ECHO parameters on detecting subclinical ATRCD and investigate the changes of LDL subfractions pattern after anthracycline therapy.

Specific objectives:

1. To determine the cumulative incidence of subclinical and clinical ATRCD in Ugandan adult cancer patients who receive anthracycline therapy.
2. To investigate the pre-existing cardiovascular risk and other relevant factors on predicting ATRCD in Ugandan adult cancer patients.
3. To determine the correlation of conventional echocardiographic parameters (MAPSE, S') with GLS, and their accuracy on detecting subclinical ATRCD in Ugandan adult cancer patients.
4. To investigate the changes of LDL subfractions pattern after anthracycline therapy in Breast cancer patients who have no pre-existing cardiovascular factors.

## **Chapter 3: Methods**

### **3.1 Study design**

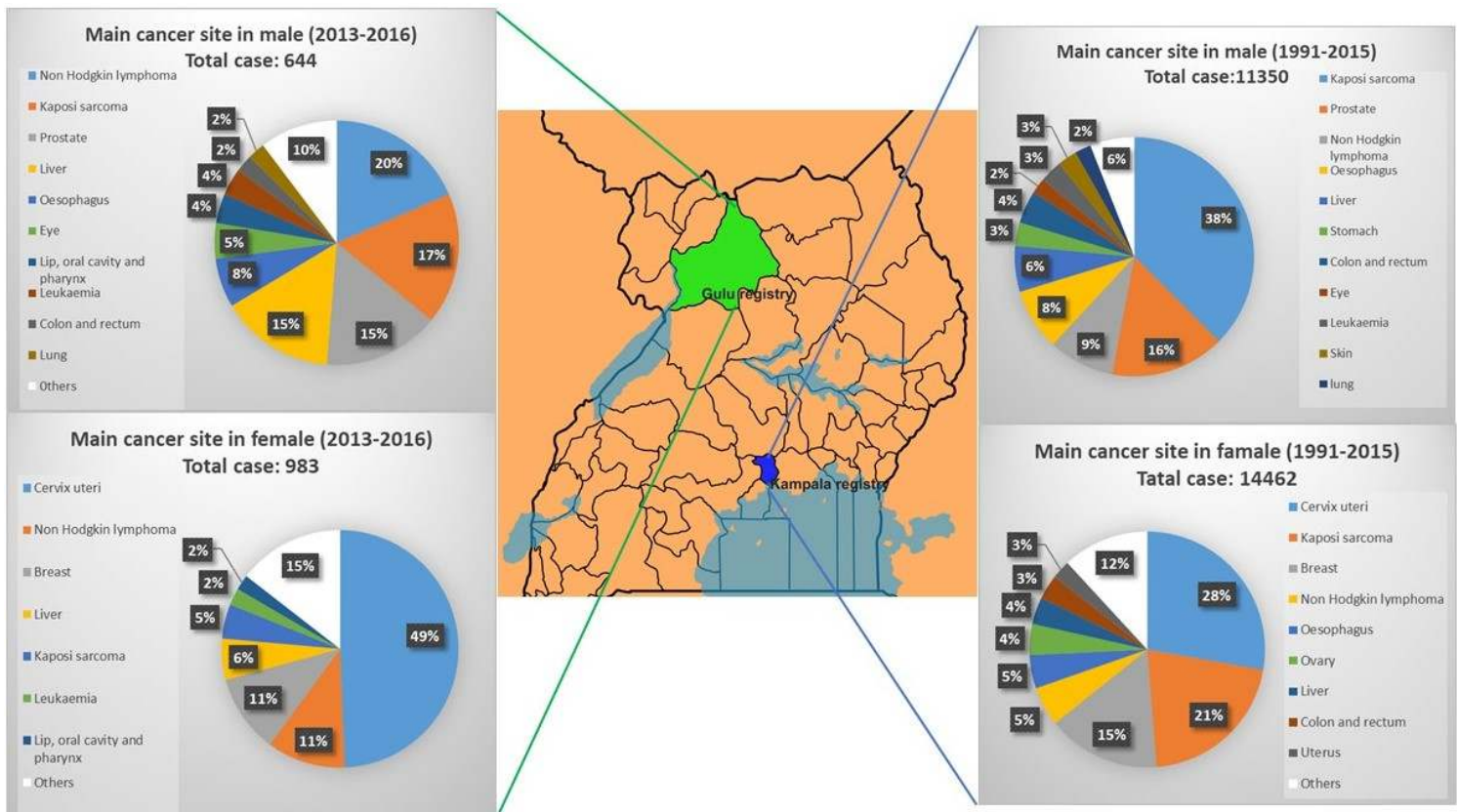
This is a prospective cohort study, consist of cancer patients who were scheduled and completed anthracycline based chemo therapy. The overall methodology of the main study is described in this chapter. The specific study methods (such as: sample size estimation, protocols for ECHO and blood test, statistical analysis...) are presented in each relevant chapters respectively.

### **3.2 Study site and population**

The study was conducted in Uganda. Patients screen site was at the UCI outpatient clinic. Cancer patients who were scheduled for anthracycline based chemotherapy were then referred to Uganda Heart Institute (UHI) for re-screening for eligibility. Patient recruitment and data collection were done at UHI.

Uganda is a low-income country in east Africa with a total population of 45.8 million people in 2021 (worldometers.info, 2022). In Uganda, about 32,617 new cancer cases and 21,829 cancer deaths were registered in 2018 (J.E.M. Ferlay et al., 2018). There are two cancer registries in Uganda. Kampala cancer registry, the first cancer registry in Uganda, was establish in 1951. It records cancer in the population of Kyadondo County, Uganda—which comprises the city of Kampala and a peri-urban hinterland (Bukirwa et al., 2021). The most common cancer registered by Kampala cancer registry are cancers of the prostate, Kaposi sarcoma in male and cervix uteri in female (Bukirwa et al., 2021). “Gulu Cancer Registry was established in 2014 to assess the incidence and survival of cancer in 4 districts of the Acholi Sub-region of northern Uganda. The most common cancers reported by Gulu registry are non-Hodgkin Lymphoma, Kaposi Sarcoma, prostate and liver cancers in male, cancers of the cervix and non-Hodgkin Lymphoma in female (Okongo et al., 2019).” **Figure 3.1** shows the map of Uganda with the geographic coverage of

the two cancer registries in the country and the cancer prevalence of the main cancer types documented.



**Figure 3. 1:** Geographic coverage of Uganda cancer registries and cancer prevalence

“UCI is the only specialized national referral oncology center in Uganda. It located in the central region of the country in Kampala capital city. The center receives about 1,000 new cancer patients referred for chemotherapy annually. Currently, it has a bed capacity of about 100 beds with 8 specialist medical oncologists, 2 pharmacists, 10 medical officers and 50 trained oncology nurses (Kibudde et al., 2019).” “UCI offers all cancer services including: prevention and early detection; diagnosis; treatment; and palliation. Cancer care at UCI is mainly offered for free including; cancer

screening; diagnosis; oncologist consultations; surgery; and all cancer medicines available in the UCI pharmacy. Due to recurrent stock-outs, patients may have to buy medicines that are not available in the pharmacy, and due to the limited number of surgical-oncologists in the country, most patients seek specialized cancer surgery from private practices. In addition, patients cost-share for radiotherapy services by paying a standard fee of about \$85USD before accessing all the prescribed radiation cycles (Nakaganda et al., 2021).” About 60% of patients attending UCI receive anthracycline therapy (Kibudde et al., 2019).”

Uganda Heart Institute is a specialized provider of cardiovascular services and the only public referral facility for heart disease in Uganda. The institute handles over 20,000 patients annually, including over 95% of the adult patients and 58% of the cases among children in Uganda.

### **3.3 Inclusion and exclusion criteria**

Inclusion criteria:

1. Age  $\geq$  18 years
2. Newly diagnosed cancer patients
3. Planned for anthracycline based chemotherapy

Exclusion criteria:

1. Impaired LV systolic function (LVEF < 50%) at the baseline<sup>#</sup>
2. Poor quality 2D image<sup>##</sup>
3. Left bundle branch block (LBBB) electrocardiogram (ECG) pattern<sup>##</sup>
4. Anthracycline therapy was changed or canceled

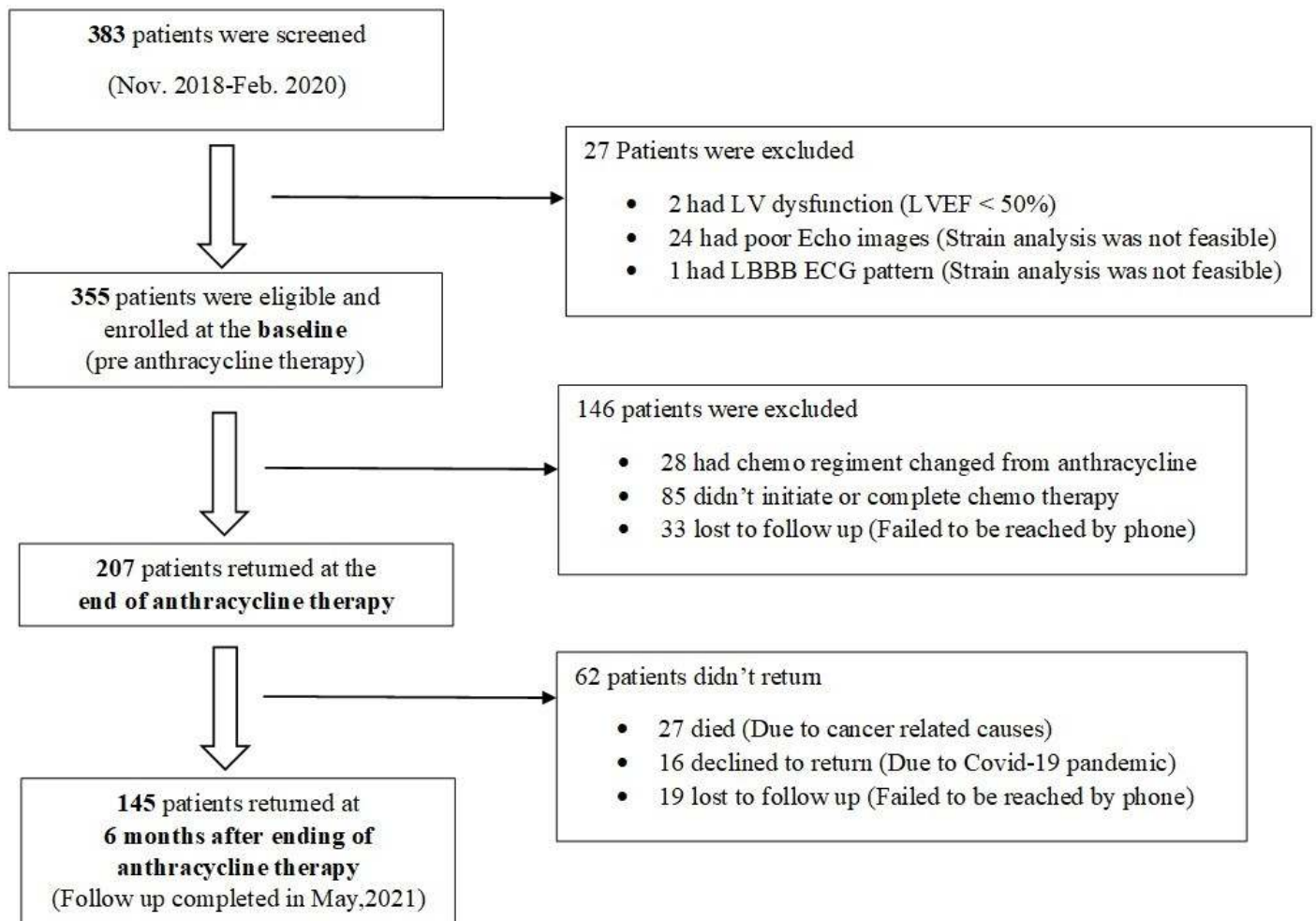
<sup>#</sup>: Patients with impaired LV function are not eligible for anthracycline chemotherapy, therefore were excluded.

##: Patients with poor quality 2D image and LBBB ECG pattern would make the correct strain analysis impossible, therefore were excluded.

### **3.4 Patients recruitment and follow up**

Between November 2018 and April 2021, adults (18 years and above) cancer patients, who were planned for anthracycline based chemo therapy, were recruited at the baseline (pre-chemo therapy) consecutively and being followed up until 6 months after ending of anthracycline therapy. Data on patient's demographics, cancer profile, past medical history were collected at the baseline. Patients' symptoms, physical examinations, ECG, ECHO and laboratory data were collected at baseline, and two follow up visits (end of anthracycline therapy and 6 months after ending anthracycline therapy) (**Table 3.1**).

A total of 383 patients were screened over a period of 15 months. Two (2) patients were excluded due to LV systolic dysfunction, 24 patients were excluded due to poor quality 2D images and 1 patient was excluded due to LBBB ECG pattern. There were 113 patients never got chemo therapy due to various reasons and 33 patients loss to follow up. In total, 207 patients enrolled at the baseline, were able to complete anthracycline therapy and came back for the 2<sup>nd</sup> visit at the end of the anthracycline therapy. Among them, 145 patients came back for the 3<sup>rd</sup> visit after 6months, 27 patients dead, 16 patients were last known alive and 19 patients were considered as lost to follow up. (**Figure 3.2**)



**Figure 3. 2:** Patients’ flow chart for the main study

### 3.5 Clinical intake protocol

The following data was collected from all enrolled subjects.

- Patient demographics: age, gender, tribe, district
- Cancer profile: Cancer diagnosis and stage, type of anthracycline and accumulated dosage\*, other medications\*
- Past medical and social history: hypertension, diabetes mellitus, coronary heart disease, renal disease, HIV, smoking, alcohol intake, any other chronic illnesses
- Family history of heart disease or cancer

- Current clinical presentation\*: symptoms of heart failure, NYHA class, heart failure stage
- Physical examination\*: weight, height, temperature, blood pressure\*\*, heart rate, SaO<sub>2</sub>, signs of heart failure (raised JVP, edema, rills...)

The following investigative tests were performed:

- 12-leads ECG\*
- Comprehensive ECHO\*: using 2D, M-mode, Doppler, tissue Doppler, STE image to assess cardiac structure, function and hemodynamics.
- Blood test\*: CBC, LFTs, RFTs, Troponin I, Lipoprotein subfractions analysis\*\*\*.

\*: Collected at each visit

\*\* : Patient’s left arm blood pressure was taken at sitting position, using “Omron” digital blood pressure machine, after resting for 5 minutes.

\*\*\*: This test was done on selected patients for sub study (Refer to chapter 7)

Data	Baseline	Completion of anthracycline therapy	6 months after completion of anthracycline therapy
Demographics	X		
Cancer data	X	X	
Medical & social history	X		
Symptoms & physical examinations	X	X	X
ECG	X	X	X
ECHO	X	X	X
Lab test	X	X	X

**Table 3. 1:** Data collection and patients follow-up

### **3.6 Informatics and Statistical Considerations**

#### **3.6.1 Study Information Management**

Study data was entered through specialized online study forms into a central database in RED Cap. RED Cap is a widely used research information system that provides for form-based entry of study data with quality assurance tools to check the validity and consistency of results.

#### **3.6.2 Data management and analysis**

Raw data was collected on the predesigned case report forms and later was entered into a study specific Redcap database for storage and further statistical analysis. We analyzed the data using Stata software (version 14). All continuous variables were expressed as a mean  $\pm$  standard deviation (SD) and categorical variables as a percentage. Statistical analysis methods to achieve the specific research objectives are presented with details in the following chapters.

### **3.7 Ethical consideration**

Ethical approvals were obtained from School of Medicine Ethics and Research Committee, College of Health Sciences Makerere University (REC REF 2018-081), Uganda National Council of Science and Technology (HS220ES) and Faculty of Health Sciences Human Research Ethics Committee, University of Cape Town (HREC 054/2020sa).

All participants were provided with a written copy of the consent form. Any and all questions that participants had regarding the research were addressed prior to signing of the consent form.

## **Chapter 4: Cardiovascular risk factors and pre-chemo strain analysis in**

### **Uganda cancer patients- Baseline data from SATRACD study**

#### **4.1 Introduction**

As in many low-middle income countries (LMICs), the growing burden of non-communicable diseases (NCDs) in Uganda is part of an epidemiologic shift catalyzed by demographic and nutritional transitions (Schwartz et al., 2014). According to the World Health Organization published data in 2018, NCDs accounted for 33% of the mortality in Uganda. Cardiovascular disease (CVD) and cancer are the two leading causes of death from NCDs in the country (Guerin et al., 2002).

The link between these two major NCDs is firmly established through shared risk factors, such as aging, tobacco use, physical inactivity, obesity, unhealthy diet, harmful use of alcohol and HIV status (Schwartz et al., 2014). Cancer can directly or indirectly cause CVDs such as pericardial effusion, infiltrative cardiac disease and venous thromboembolic phenomenon. Moreover, CVDs can result from cancer therapy, e.g. cardiac dysfunction and hypertension due to certain chemotherapy regimens (e.g. monoclonal antibodies), coronary artery disease, valvular heart disease and pericardial disease due to chest radiotherapy (Lyon et al., 2022). The link between cancer and CVDs has created the need for joint care for cancer patients by cardiologists and oncologists.

The first step to improve the cardiac care of cancer patients is by knowing the CVD risks and baseline cardiac function of this patient population. This is particularly important for patients who are planned for anthracycline therapy, as studies have suggested patients with pre-existing CVD risks (such as hypertension, diabetes mellitus, obesity, coronary heart disease...) and low baseline LVEF or GLS have higher risk of development of subclinical or clinical ATRCD (Lyon et al., 2022). Unfortunately, such data are lacking in most LMICs, including Uganda. This poses a great challenge to effective health sector planning and resource allocation. On the other hand, whether the baseline CVD risk factors and other patients'

characteristics are associated with low baseline cardiac function measured by GLS has not been study in Ugandan cancer population.

We performed baseline data analysis from this cancer cohort (SATRACD study), who were planned for anthracycline based chemotherapy, aiming to estimate the prevalence of CVD risk factors in Ugandan cancer patients, to describe the baseline strain pattern and to assess its associations with the CVD risk factors. We hypothesized that patients' CVD risk factor (s) and other patients' characteristic (s) is (are) associated with low baseline GLS in Ugandan cancer patients.

## 4.2 Method

This was a cross-sectional study from SATRACT study (a cohort of cancer patients who were planned for anthracycline based chemotherapy). Study population, baseline data collection, are described in chapter 3.

The minimum number of participants required to determine the prevalence of CVD risk factors was calculated using Kish Leslie's formula:

$$N = \frac{Z^2_{\alpha/2} p(1-p)}{d^2}$$

Where  $Z_{\alpha}$  is 1.96 for  $\alpha= 0.05$ , standard normal value at 5% two tail level of significance; d is the tolerable sampling error (precision), 5%;  $p= 0.26$ , is the prevalence of hypertension, the most common CVD risk factor reported in the latest nationwide NCDs risk factor survey in Uganda (Guwatudde et al., 2015); N is required sample size = 264.

The minimum number of participants needed to investigated the associations of variables and suboptimal GLS was calculated using Green's rule:  $N= 104 + K$ , where  $K =21$ , is the number of

independent variables. N is required sample size = 125. Therefore, the minimum number of patients required was determined by the larger sample size: 264.

#### **4.2.1 Definition of the cardiovascular risk factors**

Hypertension was defined as a systolic blood pressure (BP)  $\geq 140$  or diastolic BP  $\geq 90$  mmHg on measurements at the enrollment or self-reported current use of anti-hypertensives (Lyon et al., 2020). Diabetes mellitus, HIV status, chronic kidney disease (CKD), smoking and alcohol consumption were defined based on the patient's past medical record. Patient's weight and height were measured for calculating body mass index (BMI) and body surface area (BSA). Under weight, normal weight, overweight and obesity were defined as BMI  $< 18.5$ ,  $18.5 \leq \text{BMI} \leq 25$ ,  $25 < \text{BMI} < 30$  and BMI  $\geq 30$  respectively.

#### **4.2.2 Echocardiography protocol and equipment**

Transthoracic echocardiographic images were acquired using Vivid E9® (GE Healthcare) ultrasonography machine (**Figure 4.1**) by two cardiologists using a 1.5-4.6 MHz transducer (M5Sc). All echocardiographic images were analyzed by a single observer. LVEF was calculated using both M-mode method (from parasternal long axis view, measured manually by Teichoz's method) and 2D method (Simpson biplane, from apical 4- and 2-chamber view, measured by "automatic EF" function with necessary manual adjustment). (**Figure 4.2**) Stroke volume and cardiac index were calculated by both 2D method (apical 4- and 2-chamber views) and Doppler method (the product of LV outflow tract time velocity integral of the pulsed wave Doppler signal LV outflow tract flow area). Left atrial (LA) volume was measured using 2D biplane method (Simpson biplane) (from apical 4- and 2-chamber view) and indexed to BSA. MAPSE was measured by M-mode obtained from apical 4-chamber view (**Figure 1.6**). Average of septal and

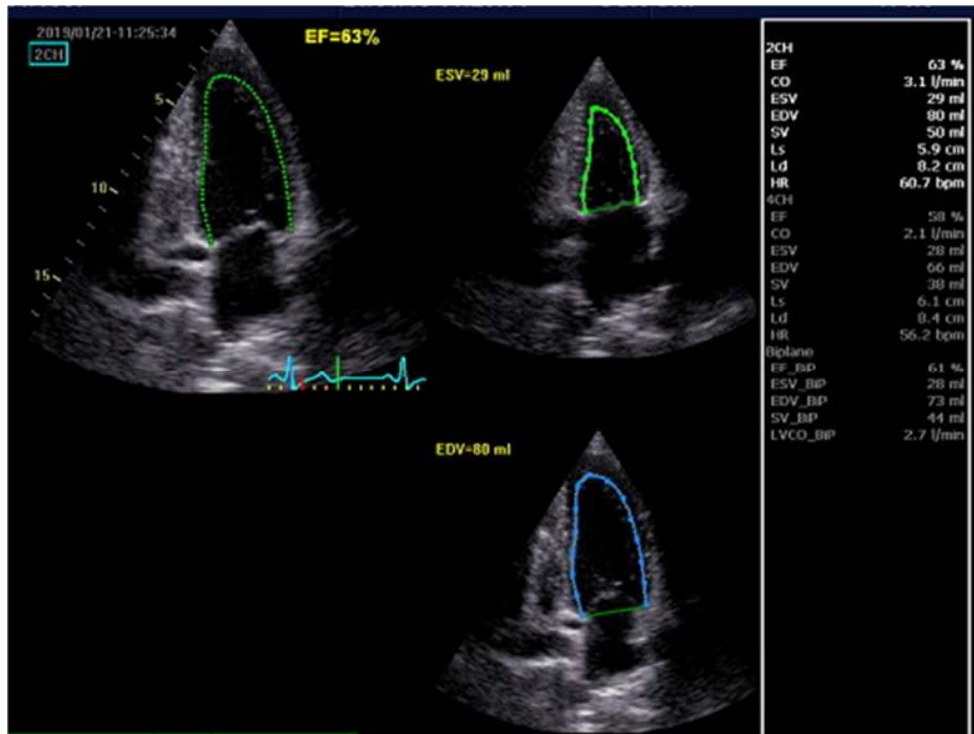
lateral MAPSE was calculated. Tissue Doppler-derived indices was measured using the apical 4-chamber view. Peak systolic mitral annular velocities (S') is calculated by averaging septal and lateral mitral annular velocities (**Figure 1.7**).

For strain analysis, automatic speckle tracking strain analysis ("AFI" function) was used to measure LV global and regional longitudinal systolic strain from apical 4-, 2- and 3-chamber views. In the case of insufficient tracking, manual correction of the endocardial tracing was attempted. Regional longitudinal strain is demonstrated on a Bull's eye diagram (**Fig 4.3**).

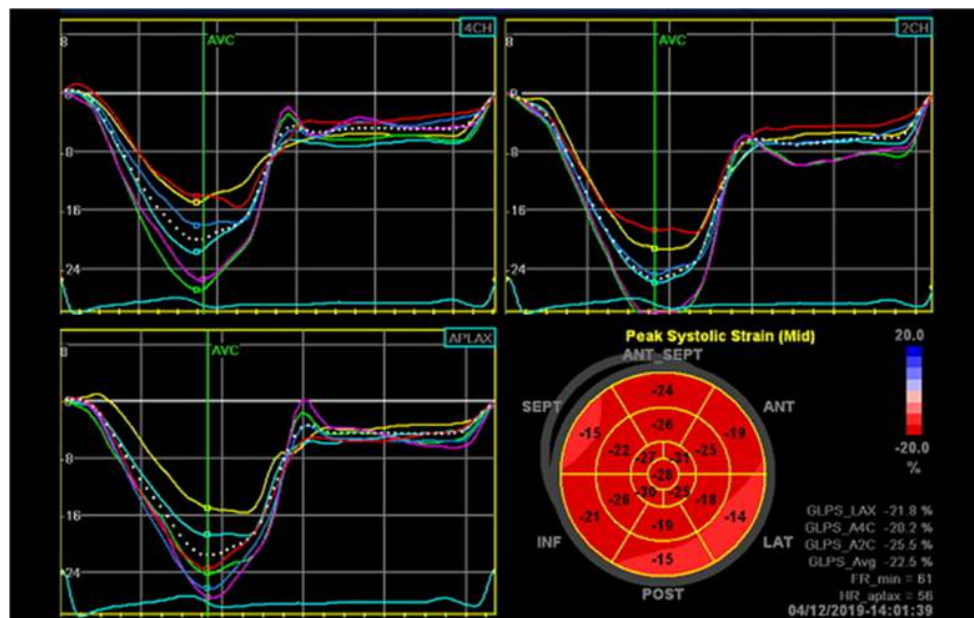
To examine inter and intra-observer reliability, we reassessed LV GLS assessments in a random sample of 10 patients. The intraclass correlation coefficients for inter and intra-observer reliability were 0.93 and 0.91 respectively for LV GLS.



**Figure 4. 1:** Transthoracic echocardiography machine used in the study: GE Vivid E9



**Figure 4. 2:** “Automatic EF” function from 2-chamber view LV ejection fraction was calculated using 2D method (Simpson biplane)



**Figure 4. 3:** Global Longitudinal Strain (GLS) Analysis

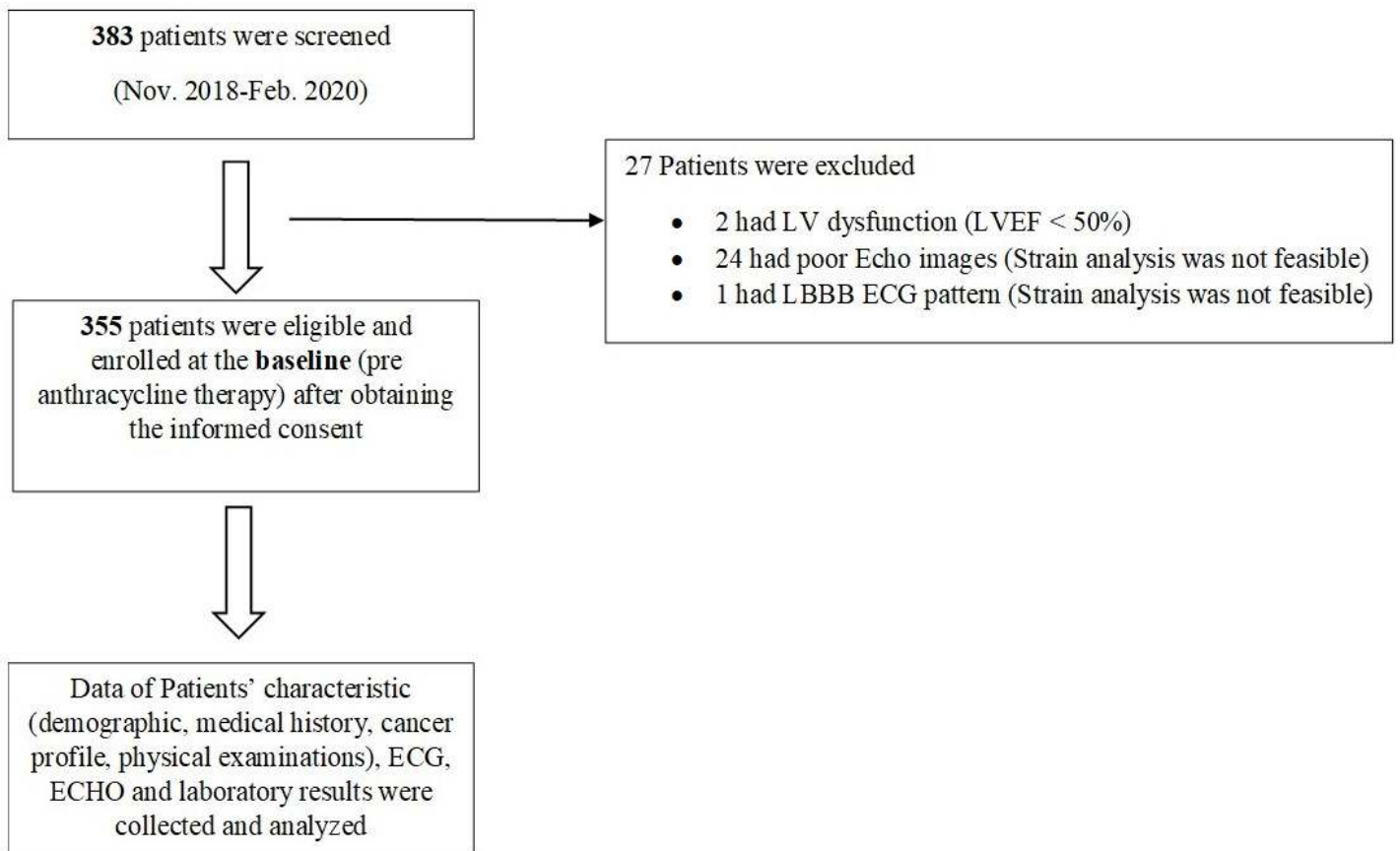
Automatic speckle tracking strain analysis (“AFI” function) was used to measure LV global and regional longitudinal systolic strain from apical 4-, 2- and 3-chamber views. Regional longitudinal strain was demonstrated on a Bull’s eye diagram.

### **4.3 Statistical analysis**

We analyzed the data using Stata software (version 14). We summarized characteristics, laboratory results, ECG and ECHO assessments for patients enrolled before receiving anthracycline therapy. All continuous variables were expressed as a mean  $\pm$  standard deviation (SD) and categorical variables as frequency and percentage. Unpaired t test with Welch correction, paired t test and ANOVA were used to compare continuous variables. Correlations of GLS and conventional ECHO parameters were assessed by Pearson's test. To assess the association between suboptimal (low) GLS and clinical characteristics, Prevalence Odds Ratio (POR) was calculated using Logistic regression. To control for the effect of the variables on the measures of association, all variables with a p-value of less than or equal to 0.2 were included in the multivariable model. A two-sided p-value  $<0.05$  was considered statistically significant for all analyses.

### **4.4 Results**

A total of 383 patients were screened over a period of 15 months. Two patients were excluded due to LV systolic dysfunction, one patient was excluded due to LBBB ECG pattern, and twenty-four patients were excluded due to poor quality 2D images. Three hundred fifty-five (355) eligible patients were recruited at the baseline (**Fig 4.4**).



**Figure 4. 4:** Patients' flow chart for baseline data analysis

#### **4.4.1 Patients' characteristics, laboratory and ECG data**

Among the 355 patients who were recruited, 283 (79.7%) were female, with a mean age of 43 years. Table 4.1 shows the clinical characteristics, laboratory and ECG data of the patients stratified by gender. Females had a significantly higher BMI, systolic BP and diastolic BP than males. There were no gender differences regarding age, body surface area, heart rate, SaPO<sup>2</sup>, laboratory and ECG parameters.

Variables	Overall (N=355)	Male (72, 20.3%)	Female (283, 79.7%)	P-value*
Age(years)	43±12	41 ± 14	43 ± 11	0.14
Physical Examination				
Body Mass Index (kg/m <sup>2</sup> )	24.7 ± 4.8	21.6±3.3	25.5 ± 4.8	<0.001
Body Surface Area (m <sup>2</sup> )	1.7 ± 0.18	1.7 ± 0.16	1.7 ± 0.19	0.49
Heart Rate (beats/min)	86 ± 16	88 ± 16	85 ± 17	0.19
Systolic BP (mmHg)	129 ± 18	124 ± 14	131 ± 19	<0.001
Diastolic BP (mmHg)	76 ± 12	74 ± 11	78 ± 12	0.008
SaPO <sub>2</sub> (%)	97 ± 2	96 ± 2	97 ± 2	0.15
Laboratory				
Hemoglobin (g/dl)	12.6 ± 2.1	12.3 ± 2.8	12.7 ± 1.9	0.30
eGFR (ml/min/1.73m <sup>2</sup> )	97.1 ± 2.1	99.8 ± 35.5	96.7 ± 30	0.67
Troponin-I (ng/ml)	0.09 ± 0.12	0.12 ± 0.19	0.09 ± 0.09	0.14
Electrocardiography				
PR interval (ms)	152 ± 22	152 ± 22	152 ± 21	0.96
QTc interval (ms)	409 ± 28	405 ± 27	410 ± 28	0.19

**Table 4. 1:** Patients characteristics, laboratory and ECG data

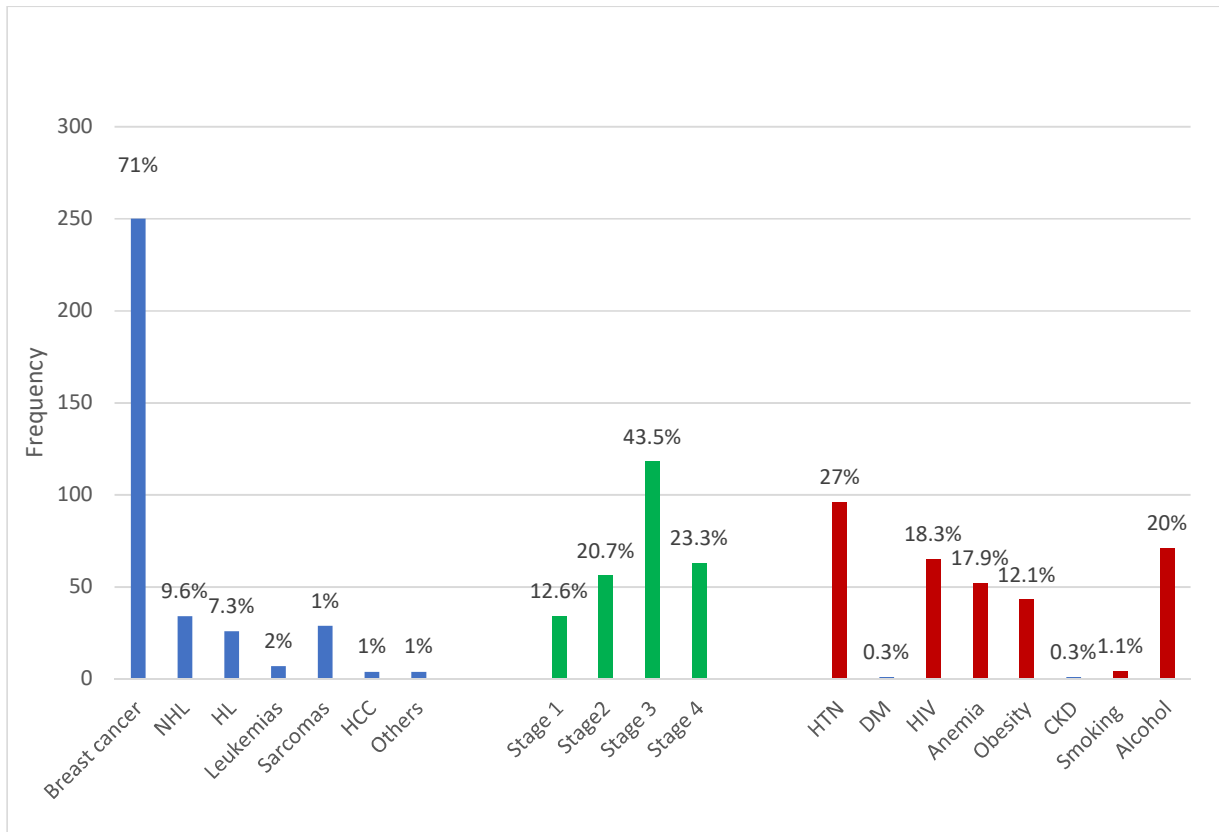
\*: *P* value derived from unpaired t-test with Welch correction

#### 4.4.2 Patients' cancer profile and cardiovascular risk factors

Figure 4.5 shows the cancer type, staging and cardiovascular risk factors distribution of the study population. Breast cancer was the most commonly diagnosed cancer (250, 70.6%) in the group, followed by non-Hodgkin's lymphoma (34, 9.6%), sarcomas (29, 8.2%), Hodgkin's lymphoma (26, 7.3%), leukemia (7, 2.0%), hepatocellular carcinoma (4, 1.1%) and others (4, 1.1%). Most patients were found to have stage 3 (43.5%) and stage 4 (23.3%) diseases. Hypertension (27.0%) was the most common cardiovascular risk factor seen in the patients, followed by alcohol use (20.0%), HIV (18.3%), anemia (17.9%), obesity (12.1%), smoking (1.1%), Type 2 diabetes mellitus (0.3%) and CKD (0.3%).

Non-Hodgkin's lymphoma was the only AIDS defining cancer. There was no difference regarding the HIV positive patients' distribution between AIDS defining cancer and non AIDS defining

cancer (8, 21.6% vs 57, 17.4%;  $p= 0.255$ ). There were more patients presented with obesity in breast cancer group than other cancer patients (41,16.5% vs 7, 6.7%;  $p= 0.017$ ).



**Figure 4. 5:** Cancer profile and cardiovascular risk factors

NHL: non-Hodgkin’s lymphoma, HL: Hodgkin’s lymphoma, HCC: hepatocellular carcinoma, HT: hypertension, DM: diabetes mellitus, CKD: chronic kidney disease.

#### 4.4.3 Conventional and strain ECHO measurements

**Table 4.2** shows the conventional ECHO measurements and longitudinal strain ECHO measurements stratified by gender. For all the conventional ECHO parameters, male participants had significantly larger chamber sizes than females, except for LA volume. Male participants also had higher  $E'$ , stroke volume (SV), cardiac index (CI) and lower  $E/E'$  ratio than women. There was no difference in LV systolic function assessed by LVEF between male and female

participants. The LVEF measured by two methods showed significant difference, with higher value by M- mode than by 2D method. The SV and CI assessed by two methods also showed significant difference, with higher value obtained by Doppler method than 2D method.

For strain analysis, the mean GLS for the study population was  $-20.9 \pm 2.43\%$ , with males having significantly lower absolute GLS levels than females. Segmental strain analysis found that there was no difference among longitudinal strain in apical 3 chamber view, apical 4 chamber view and apical 2 chamber view. There was significant longitudinal strain difference between the basal, mid and apical regions, with the basal region having the lowest absolute strain and apical the highest absolute strain.

Variables	Overall (N=355)	Male (N=72)	Female (N=283)	P value
LVDD (mm)	44.87 ± 4.85	47.22 ± 4.16	44.27 ± 4.84	<0.001
LVEDV (ml)	68.07 ± 18.18	81.43 ± 21.73	64.67 ± 15.45	<0.001
LVEDVI (ml/m <sup>2</sup> )	39.77 ± 9.96	46.98 ± 11.42	37.90 ± 8.64	<0.001
LVEF (M-Mode) (%)	68.75 ± 6.6	67.97 ± 5.8	68.94 ± 6.79	0.223
LVEF (2D) (%)	60.97 ± 5.66	60.93 ± 4.95	60.98 ± 5.83	0.943
	<b>P&lt;0.001<sup>†</sup></b>			
MAPSE (mm)	14.49 ± 2.14	14.55 ± 2.17	14.48 ± 2.14	0.783
LAV (ml)	25.65 ± 9.74	27.39 ± 11.45	25.21 ± 9.23	0.138
LAVI (ml/m <sup>2</sup> )	14.93 ± 5.38	15.78 ± 6.37	14.72 ± 5.08	0.195
RVD (mm)	24 ± 2.94	26 ± 3.22	23.85 ± 2.71	<0.001
RAA (cm <sup>2</sup> )	10.9 ± 2.52	12.2 ± 2.75	10.58 ± 2.36	<0.001
TAPSE (mm <sup>2</sup> )	24.37 ± 3.98	24.37 ± 4.1	24.37 ± 3.96	0.999
E (cm/s)	81.49 ± 17.34	79.39 ± 18.75	82.04 ± 16.95	0.277
E/A	1.15 ± 0.37	1.24 ± 0.47	1.12 ± 0.34	0.062
E' (cm/s)	12.22 ± 3.07	13.11 ± 3.43	12 ± 2.94	0.013
S' (cm/s)	9.62 ± 2.06	10.56 ± 2.08	9.38 ± 1.99	<0.001
E/E'	7.01 ± 2.04	6.32 ± 1.68	7.19 ± 2.09	<0.001
SV (2D) (ml)	41.65 ± 11.64	49.35 ± 14.2	39.70 ± 10.02	<0.001
SV (Doppler) (ml)	59.82 ± 15.91	65.64 ± 16.01	58.36 ± 15.58	<0.001
	<b>P&lt;0.001*</b>			
CI (2D) (ml/L/m <sup>2</sup> )	2.05 ± 0.59	2.39 ± 0.66	1.96 ± 0.55	<0.001
CI (Doppler) (ml/L/m <sup>2</sup> )	2.87 ± 0.80	3.08 ± 0.82	2.81 ± 0.79	0.014
	<b>P&lt;0.001<sup>#</sup></b>			
GLS (%)	-20.92 ± 2.43	-20.25 ± 2.39	-21.09 ± 2.42	0.009
LAX (%)	-20.74 ± 3.19	-20.13 ± 3.29	-20.90 ± 3.16	0.081
A4C (%)	-20.50 ± 2.91	-19.8 ± 2.32	-20.67 ± 3.01	0.011
A2C (%)	-21.50 ± 3.05	-20.70 ± 2.83	-21.70 ± 3.07	0.011
	<b>P=0.208<sup>+</sup></b>			
Base (%)	-17.05 ± 2.82	-16.39 ± 2.99	-17.22 ± 2.75	0.034
Mid (%)	-20.10 ± 2.48	-19.53 ± 2.50	-20.25 ± 2.46	0.031
Apex (%)	-25.61 ± 3.88	-24.96 ± 3.52	-25.77 ± 3.96	0.091
	<b>P&lt;0.001<sup>++</sup></b>			

**Table 4. 2:** Conventional and Strain echocardiography measurements

LVDD: left ventricle diastolic diameter, LVEDV: left ventricle end diastolic volume, LVEDVI: left ventricle end diastolic volume index, LVEF (M-Mode): M-Mode left ventricular ejection fraction, LVEF (2D): 2D left ventricular ejection fraction, MAPSE: mitral annular plane systolic exertion, LAV: left atrium volume, RVD: right ventricular diameter, RAA: right atrium area, TAPSE: tricuspid annular plane systolic exertion, E: left ventricle inflow pulse wave Doppler E wave, E': tissue Doppler mitral annular diastolic E' velocity, S': tissue Doppler mitral annular systolic velocity, SV (2D):2D stroke volume, SV (Doppler): Doppler stroke volume, CI (2D): 2D cardiac index, CI (Doppler):Doppler cardiac index, GLS: global longitudinal strain, LAX: apical long axis view A4C:apical 4 chamber view, A2C: apical 2 chamber view  
‡: P value compare LVEF measured by M-Mode and 2D biplane method  
\*: P value compare SV measured by 2D biplane method and Doppler method.  
#: P value compare CI measured by 2D biplane method and Doppler method  
+: P value compare segmental longitudinal strain at apical long axis view, apical 4 chamber view and apical 2 chamber view  
++: P value compare segmental longitudinal strain at the base, mid and apex of the left ventricle.

**Table 4.3** shows the correlation between absolute GLS and conventional ECHO parameters. We found absolute GLS positively correlated with many conventional ECHO parameters. Among the conventional parameters, 2D LVEF had the best correlation with GLS follow by MAPSE lateral.

GLS (%)	<i>r</i>	<i>P</i> value
LVEF (Mmod)	0.262	<0.001
LVEF (2D)	0.592	<0.001
MAPSE	0.368	<0.001
MAPSE (Medial)	0.294	<0.001
MAPSE (Lateral)	0.388	<0.001
S'	0.225	<0.001
S' (Medial)	0.232	<0.001
S' (Lateral)	0.180	0.0008
E	0.240	<0.001
E'	0.199	0.0002
E' (Medial)	0.199	0.0007
E' (Lateral)	0.182	0.0002
LVOT VTI	0.225	<0.001
SV (2D)	0.145	0.0063
SV (Doppler)	0.038	0.4863

**Table 4. 3:** Correlation of GLS and conventional Echo parameters

LVEF (Mmod): Mmod left ventricle ejection fraction, LVEF (2D): 2D left ventricle ejection fraction, MAPSE: mitral annular plane systolic exertion, S': tissue Doppler mitral annular systolic velocity, E: left ventricle inflow pulse wave Doppler E wave, E': tissue Doppler mitral

annular diastolic E' velocity, LVOT VTI: velocity time integral of left ventricular out flow track, SV (2D): 2D stroke volume, SV (Doppler): Doppler stroke volume

#### **4.4.4 Association of low GLS and patient's characteristics**

We defined suboptimal GLS (low GLS) as absolute GLS  $\leq$  18.00% (Yang et al., 2018). There were 53 (14.9%) patients were found to have low GLS. Table 4.4 shows the association analysis between patients' clinical characteristics, cardiovascular risk factors, ECG and ECHO findings with low GLS. On bivariate analysis, we found low GLS associated with obesity, Alcohol use, QTc>440ms and impaired LV relaxation. After adjusting for patients' sex, HIV status, alcohol use, overweight, obesity, QTc>440ms on ECG and impaired LV relaxation, obesity was the only independent factor associated with low GLS.

Variables(N)	Low GLS ( Total N=53) N(%)	Bivariate models		Multivariable model	
		POR(95%CI)	P-value	POR(95%CI)	P-value
Female(283)	37 (13.07%)	0.53 (0.26-1.09)	0.052	0.54 (0.22-1.33)	0.17
Age>60 years(32)	4 (12.50%)	0.80 (0.19-2.43)	0.69		
Hypertension(96)	14 (14.58%)	1.11 (0.52-2.22)	0.77		
HIV(65)	14 (21.54%)	1.77 (0.82-3.61)	0.098	1.08 (0.41-2.83)	0.87
Alcohol (71)	16 (22.53%)	1.94 (1.01-3.74)	0.044	1.59 (0.93-2.71)	0.94
Tachycardia(66)	9 (13.64)	1.67 (0.65-3.87)	0.21		
Normal BMI(184)	27 (14.68%)	1			
Under Weight(21)	5 (23.81%)	1.98 (0.52-6.34)	0.21		
Over Weight(102)	7 (6.86%)	0.47 (0.16-1.17)	0.084	0.52 (0.21-1.32)	0.11
Obesity(43)	14 (32.56%)	3.07 (1.31-6.98)	0.003	2.95 (1.24-7.03)	<b>0.014</b>
Hematological Malignancy(71)	14 (19.72%)	1.43 (0.82-2.49)	0.21		
Stage 1 (34)	4 (11.76%)	1			
Stage 2 (56)	11 (19.64%)	1.83 (0.48-8.59)	0.33		
Stage 3 (117)	14 (11.96%)	1.02 (0.29-4.57)	0.97		
Stage 4 (63)	13 (20.63%)	1.95 (0.53-8.91)	0.27		
No Anemia(210)	30 (14.28%)	1			
Mild Anemia* (63)	12 (19.05%)	1.44 (0.62-3.15)	0.33		
Mod- severe Anemia** (18)	3 (16.67%)	1.20 (0.21-4.62)	0.78		
Troponin>0.3ng/ml (6)	1 (16.67%)	1.02 (0.02-9.40)	0.98		
QTc>440ms(40)	11 (27.50%)	2.54 (1.06 -5.74)	0.015	2.51 (0.95-6.62)	0.062
Impaired LV relaxation(84)	24 (28.57%)	2.24 (1.17-4.25)	0.007	2.10 (0.97-4.58)	0.060

**Table 4. 4:** Association of low GLS and variables

POR: Prevalence Odds Ratio

\*: Hemoglobin 10.0 g/dL to lower limit of normal (13.5 g/dL in men;12.0 g/dL in women)

\*\* : Hemoglobin < 10.0g/dL

## 4.5 Discussion

### 4.5.1 Cardiovascular risk factors in Uganda cancer patients

In this study, we assessed cardiovascular risk factors and pre-chemo cardiac function using strain imaging among cancer patients planned for anthracycline chemotherapy. Female patients and patients with breast cancer accounted for the majority of patients studied. In this group of cancer patients, we observed all the cardiovascular risk factors which are present in the general

population, including hypertension, diabetes mellitus, obesity, CKD, smoking, drinking alcohol, anemia and HIV, among which hypertension is the most prevalent cardiovascular risk factor. These are the same findings reported in other cancer populations (Blanco JG et al., 2012). This result shows early diagnosis and treatment of hypertension in cancer patients is essential because hypertension is a major risk factor for the development of chemotherapy-induced cardiotoxicity (Suter and Ewer, 2012). In addition, suboptimal blood pressure control may lead to premature discontinuation of chemotherapy, thus affecting cancer therapy directly (Suter and Ewer, 2012, Abi Aad et al., 2015).

The latest nationwide NCDs risk factor survey in Uganda, with a younger mean age compared to our study population (31years vs 43years), revealed prevalence of hypertension, diabetes mellitus, obesity and alcohol use which were 26.5%, 1.4%,6.4% and 27.0% respectively (Guwatudde et al., 2015, Bahendeka et al., 2016). We found a similar prevalence of hypertension (27.0%) and alcohol use (20.0%) in our study patients. The lower prevalence of diabetes mellitus (0.3%) in our results could be due to a different study definition of the diabetes mellitus, whereby past medical records were used in our study rather than blood sugar testing. This difference may also suggest a potentially undiagnosed diabetes mellitus group in the community. More interestingly, obesity prevalence (12.1%) was strikingly higher in this cancer cohort than in the local population. Such result marks the fact that obesity as a traditional CVD risk factor promotes cancer as well, and breast cancer has been recognized as one of the obesity-associated cancers (Iyengar et al., 2016). This has been reflected in our results which showed higher proportion of obesity among breast cancer patients compared to non-breast cancer patients. There is growing evidence that inflammation is a central and reversible mechanism through which obesity promotes cancer risk and progression (Iyengar et al., 2015). Locally, hyper

adiposity is associated with altered adipose tissue function, adipocyte death, and chronic low-grade inflammation (de Visser et al., 2006). Most individuals who are obese harbor inflamed adipose tissue, which resembles chronically injured tissue, with immune cell infiltration and remodeling (de Visser et al., 2006). Within this distinctly altered local environment, several pathophysiologic changes are found that may promote breast and other cancers. Systemically, the metabolic syndrome, including dyslipidemia and insulin resistance, occurs in the setting of adipose inflammation and operates in concert with local mechanisms to sustain the inflamed microenvironment and promote tumor growth (Monteiro and Azevedo, 2010).

HIV is another cancer promoter, which accounts for a higher prevalence in the study patients than in general population (18.3% vs 6.5%). The most dramatic increase in cancers in Uganda has been noted due to the HIV epidemic in the country (Parkin et al., 1999). While obesity has been a traditional risk factor for CVD, recent evidence suggests that HIV-associated inflammation and immune activation are important mediators of cardiovascular risk (Triant, 2013). The interaction among HIV, cancer and cardiac disease is discussed further in chapter 5.

#### **4.5.2 Conventional ECHO patterns in Uganda cancer patients**

Despite the burden of cardiovascular risk factors in this study group, they all have normal LV systolic function defined by LVEF at baseline. In order to detect subclinical cardiac dysfunction after anthracycline therapy, each patient was assessed with both conventional ECHO and speckle tracking image for strain analysis at baseline. Given that all patients had normal LVEF, do they all have normal strain values and what are the associations between their clinical characteristics and strain value? We therefore analyzed baseline ECHO data aiming to answer these questions.

Regarding the conventional ECHO assessment, all the patients had normal chamber size, with male had bigger chamber size and higher stroke volume than female. There was no gender difference for LVEF. These findings agree with the data generated from international study based on healthy population (Kou et al., 2014). For the first time in our population, we compared certain ECHO parameters measured by two different methods. The result comparing LVEF measured by M-mode and 2D ECHO (Simpson's biplane) showed M-mode LVEF, which is routinely used on our daily practice, has significant higher value than 2D LVEF. Evidence has shown that 2D ECHO LVEF have a good agreement with cardiac MRI, which is the gold standard measurement for LVEF and LV volume (Hoffmann et al., 2005). This finding indicates M-mod method can overestimate LVEF even in the heart without structure abnormalities. Although 2D LVEF has been recommended by the current international guidelines (2016), M-mode LVEF is still being commonly used in our setting due to its advantage of less time consuming and less image quality dependent. According to our findings, to balance the advantages and pitfalls of M-mode LVEF, in clinical setting, we suggest to combine this method with visual assessment and using 2D LVEF on selected individuals who has structure heart diseases. In research settings and for patients follow up, we strongly recommend 2D LVEF to be used. We also compared the SV measured by 2D ECHO (Simpson's biplane) and LV outflow tract (LVOT) pulse wave Doppler method, 2D SV is significantly lower than Doppler SV. We noted the scarcity of data to compare these two SV means. Further robust research is need to assess the validity of SV ECHO measurements with gold standard method.

#### **4.5.3 Pre chemo strain pattern in Uganda cancer patients**

Longitudinal strain analysis by speckle tracking is a novel ECHO modality, which has been increasingly utilized in both clinical and research settings, including cardio-oncology practice.

This is largely due to several advantages of this technique, such as higher sensitivity to detect cardiac dysfunction, good reproducibility and less volume dependent (Plana et al., 2014). Our baseline pre-chemotherapy strain analysis showed the mean GLS value of  $-20.92 \pm 2.43\%$ , with females having higher absolute GLS level than males. These results are in line with the findings in healthy populations (Takigiku et al., 2012) and similar cancer patients (Mornoş and Petrescu, 2013), with the same vendor-Vivid E9 (GE Healthcare). The longitudinal strain measured from apical 3 chamber view, apical 4 chamber view and apical 2 chamber view did not show significant difference. However, the mean apical, mid and basal longitudinal strain showed stepward strain gradient, with apical region having the highest longitudinal strain. This pattern has been consistently observed in several studies (Takigiku et al., 2012, Xia et al., 2020, Yuan et al., 2014). Compared to the base, the apex is smaller and is subject to less wall stress, which may result in relatively higher longitudinal strain (Bogaert and Rademakers, 2001).

Despite the advantages of speckle tracking image, several reasons are impeding its application in our settings. Indeed, many cardiologists are not trained using this methodology, and this advanced technology comes with expensive equipment that is not widely available in the country. When speckle tracking image is not available, conventional ECHO parameters, which measure the longitudinal motion of the left ventricle (e.g. MAPSE,S'), may potentially be useful for detecting subclinical cardiac dysfunction (Plana et al., 2014). However, their correlations with GLS have not been studied. Therefore, we performed Pearson's test to calculate the correlation between GLS and conventional Echo parameters. Our results demonstrated significant correlations of GLS with many conventional ECHO parameters. Among them, the best correlation parameters were 2D LVEF and MAPSE lateral, with the correlation coefficients of 0.593 and 0.388 respectively. These data have shed light on the roles of the conventional

ECHO on detecting subclinical cardiac dysfunction after anthracycline therapy. Our longitudinal data has been able to address this question in chapter 6.

#### **4.5.4 Association of CVD risk factors and pre chemo longitudinal strain**

In this study, although all the patients had normal LVEF, 53 (14.9%) patients were defined to have suboptimal absolute GLS (low GLS) of  $\leq 18.00\%$  (Yang et al., 2018). We did not find association of low GLS with gender, age, hypertension, as found by other studies (Kuznetsova et al., 2008, Narayanan et al., 2009). Furthermore, factors such as HIV, anemia, tachycardia, cancer type and cancer stages were not associated with low GLS. Interestingly, obesity was the only independent factor associated with low GLS in our study. As we have discussed above, obesity, as a shared risk factor for cancer and cardiovascular disease, is not only found to have a high prevalence in this cancer cohort, but also related to low GLS. This has also been revealed by Wong C. Y and their colleges (Wong et al., 2004). They studied 109 overweight or obese subjects and 33 referents (BMI  $< 25 \text{ kg/m}^2$ ) and found that the obese subjects (BMI  $> 35 \text{ kg/m}^2$ ) had reduced LV systolic and diastolic function, compared with referents (evidenced by lower average long-axis strain), whereas LVEF remained normal. It has been postulated that obesity leads to cardiac dysfunction through several direct and indirect mechanisms, including hemodynamic changes, myocardial fat accumulation, inflammatory cytokines, dyslipidemia and other comorbidities, e.g. diabetes mellitus, obstructive sleep apnea (Csige et al., 2018). Larger cancer cohort study will help us to investigate on the impact of obesity on cancer patient's outcomes, particularly on cardiovascular outcomes of cancer survivors.

#### **4.6 Limitations**

Our study is the first study to describe cardiovascular risk factors and pre-chemotherapy strain patterns in Ugandan cancer patients, although it has several limitations.

Being a cross sectional data per se, it always implies the intrinsic limitation determined by the nature of the study design. Our research patients, who were primarily selected for anthracycline therapy in a single health center, were composed of certain cancer types with predominately female gender. Therefore, the findings may not be generalized for the whole cancer population in Uganda. Due to the limitation of research funds, some of the laboratory test for CVD risk assessment, such as lipid profile, HbA1C, were not done on research patients. We also realized the potential bias may exist due to unmeasured or unadjusted confounders. Lastly, the ECHO observers were not blinded to patients' status - this may lead to potential measurement bias. However, automatic software was used for the strain analysis and 2D LVEF measurement, which we think can minimize the bias.

#### **4.7 Conclusion**

We found a high prevalence of cardiovascular risk factors in cancer patients, with hypertension being the most common, and a much higher prevalence of obesity and HIV than in the general population. Most patients had a normal strain pattern at the baseline. Some cancer patients, GLS is reduced, despite preserved LVEF, in the presence cardiovascular risk conditions such as obesity. Pre-existing suboptimal GLS and its associated risk factors could have important implications in cancer care. It is reasonable to assume that individuals with low GLS and associated risk factors at baseline will be more susceptible to chemotherapy related cardiac dysfunction and adverse outcomes. Therefore, longitudinal research is needed to study these hypotheses.

## Chapter 5: Cumulative incidence and predictors of subclinical and clinical ATRCD in patients attending Uganda Cancer Institute- Longitudinal data from SATRACD study

### 5.1 Introduction

In the previous chapter we have presented the patients baseline data, focusing on patients pre-existing CVD risk factors, baseline strain analysis and the associations between the patients CVD risk factors and the low absolute GLS ( $\leq 18\%$ ). We found hypertension, obesity and HIV infection are the most prevalent CVD risk factors in the study population and obesity is independently associated with low GLS. Whether these pre-existing CVD risk factors and low GLS imply a higher risk of developing anthracycline cardiotoxicity can be better addressed by a longitudinal study.

In this chapter, we present and discussed the results of the longitudinal data collected at baseline (pre anthracycline therapy), 2<sup>nd</sup> visit (the end of anthracycline therapy) and 3<sup>rd</sup> visit (6 months after ending anthracycline therapy), in order to fulfill the objective 1 and 2 of this PhD project:

Objective 1: To determine the cumulative incidence of subclinical and clinical ATRCD in Ugandan cancer adult patients who receive anthracycline therapy.

Objective 2: To investigate patients' pre-existing CVD risk factors and other relevant factors on predicting ATRCD in Ugandan adult cancer patients who receive anthracycline therapy.

We hypothesize that pre-existing cardiovascular risk factors, including hypertension, obesity and HIV infection predict the development of cardio toxicity in Ugandan cancer patients treated with anthracycline.

## 5.2 Method

The detailed description of the study design, patient selection, and data collection have been described in chapter 3.

The minimum number of participants required to determine the incidence of subclinical ATRCD was calculated using Kish Leslie's formula:

$$N = \frac{Z_{\alpha/2}^2 p(1-p)}{d^2}$$

Where  $Z_{\alpha}$  is 1.96 for  $\alpha = 0.05$ , standard normal value at 5% two tail level of significance;  $d$  is the tolerable sampling error (precision), 5%;  $p = 0.22$ , is the incidence of subclinical ATRCD among cancer patient in Anita Boyd study (Boyd et al., 2017);  $N$  is required sample size = 264.

Considering loss to follow up of 10% and early death, we increased the sample size to 355 patients.

The minimum number of participants needed to investigate the predictors of ATRCD was calculated using Green's rule:  $N = 104 + K$ , where  $K = 19$ , is the number of independent variables.  $N$  is required sample size = 123. Therefore, the minimum number of patients required was determined by the larger sample size: 355.

Between November 2018 and April 2021, 355 patients were recruited at the baseline (pre-chemo therapy). Among them, 207 patients completed anthracycline therapy and were followed up until 6 months after ending of anthracycline therapy (**Figure 3.1**). At baseline, patient's demographics, cancer profile and past medical history were recorded. Patients' symptoms, physical examinations, ECG, echocardiographic and laboratory data were collected at baseline, and two follow up visits (end of anthracycline therapy and 6 months after ending anthracycline therapy).

### **5.2.1 Anthracycline and its administration**

Nonliposomal doxorubicin (traditional formulation) was administered to the patients by slow intravenous infusion. Cumulative anthracycline dose was indexed by body surface area and recorded at the end of the chemotherapy.

### **5.2.2 TnI measurement**

In all patients, 10ml of blood samples were obtained from venal puncture after 12 hours fasting. The blood samples were collected in EDTA tubes and SST tubes and immediately centrifuged. Serum TnI levels were analyzed immediately at UHI laboratory by an immunoassay (Ichroma II) with the normal range of 0.0 to 0.3ng/ml. The rest of plasma and serum were stored at -80 degree Celsius until assayed. The stored blood samples were transported in dry ice and shipped to the Cape Heart Institute, University of Cape Town, where the assay for LDL subfractions was carried out for the lipid sub study (Refer to chapter 7).

### **5.2.3 Definition of subclinical ATRCD & clinical ATRCD**

Subclinical ATRCD and clinical ATRCD were diagnosed using American Society of Echocardiography and the European Association of Cardiovascular Imaging criteria (Plana et al., 2014), outlined below:

Diagnostic criteria for subclinical ATRCD:

- LVEF  $\geq 50\%$  and a relative percentage decrease of GLS  $\geq 15\%$ , compared with baseline, and/or troponin-I becoming positive during follow-up.

Diagnostic criteria for clinical ATRCD:

- Decrease in LVEF of  $>10$  percentage points, to a value of  $<50\%$ .

### **5.3 Statistic analysis**

We analyzed the data using STATA v14 (Institute Inc., College Station, TX, USA). All continuous variables were expressed as a mean  $\pm$  SD and categorical variables as frequencies and percentages. Cumulative incidence of subclinical and clinical ATRCD were calculated using Aalen-Johansen estimator to take into account competing event. One-way ANOVA (for Gaussian distributed data) and Kruskal-Wallis test (for non-Gaussian distributed data) with post hoc analysis (2x2 comparison with Bonferroni correction) were used to compare the independent continuous variables. One-way repeated ANOVA (for Gaussian distributed data) with post hoc analysis (pairwise comparisons using Tukey's test), Friedman's ANOVA (for non-Gaussian distributed data) with post hoc analysis (pairwise Wilcoxon signed rank test), were used to compare dependent continuous variables, where appropriate. Chi-square test and Fisher's exact test were used to compare the categorical variables. To identify the predictors of subclinical and clinical ATRCD, Hazard Ratio (HR) were calculated using competing risk regression with Fine-Gray modification. To control for the effect of the variables on the measures of association, all variables with a *p*-value of less than or equal to 0.2 were included in the multivariable model to predict subclinical ATRCD. A two-sided *p*-value  $<0.05$  was considered statistically significant for all analyses.

### **5.4 Results**

#### **5.4.1 Patients' clinical characteristics**

In total, 207 patients enrolled at the baseline, were able to completed anthracycline therapy and came back for the 2<sup>nd</sup> visit at the end of the anthracycline therapy. Among them, 145 patients came back for the 3<sup>rd</sup> visit after 6months, 27 patients dead, 16 patients were last known alive and 19 patients were considered as loss of follow up (**Figure 3.1**). All the deaths were owing to cancer-related causes.

Patient's laboratory, ECG, conventional and strain ECHO data at baseline and each follow-up visit are summarized in **Table 5.1**. During follow up visits, there were significant reduction of LVEF, MAPSE, tricuspid annular plane systolic excursion (TAPSE), stroke volume, GLS and all the regional longitudinal strain. Post-hoc analysis shows these significant changes mainly occurred when comparing the 2<sup>nd</sup> visit vs baseline and 3<sup>rd</sup> visit vs baseline. There was significant increase in troponin I in 3<sup>rd</sup> visit compare to baseline. Interestingly, there was significant reduction of the hemoglobin level at the end of the anthracycline therapy. But, after 6 month of ending anthracycline therapy, the hemoglobin level significantly raised.

At the end of follow-up, a lower LVEF and lower GLS than baseline was also observed in patients who did not develop subclinical or clinical ATRCD (LVEF from 60.9±6% to 58.8±7%;  $p=0.009$ . GLS from -20.9±2% to -20.2±2%;  $p=0.04$ ).

Variables (mean±SD)	Baseline (N=207)	2nd Visit (N=207)	3rd Visit (N=145)	p value	Post-hoc analysis					
					2nd visit Vs Baseline		3rd visit Vs Baseline		3rd visit Vs 2nd Visit	
					Mean difference	p value*	Mean difference	p value*	Mean difference	p value*
<b>Laboratory</b>										
Hb(g/dl)	12.80±1.93	11.95±1.69	12.51±1.66	<0.001	-0.86	<0.001	-0.28	0.159	0.57	<0.001
eGFR(ml/min/1.73m <sup>2</sup> )	102.24±30.62	102.99±34.38	110.14±36.01	0.2393						
Troponin-I(ng/ml)	0.09±0.14	0.15±0.19	0.18±0.32	0.005	0.05	0.063	0.79	0.006	0.028	0.823
<b>Electrocardiogram</b>										
PR(ms)	153.01±22.36	152.75±20.54	153.78±22.19	0.9399						
QTc(ms)	409.88±29.70	409.83±38.82	405.66±35.65	0.1337						
<b>Echocardiography</b>										
LVEDV(ml)	67.50±16.22	67.65±17.58	70.08±15.73	0.1366						
LVEF(%)	60.92±5.78	57.54±6.18	56.51±8.55	<0.001	-3.42	<0.001	-4.17	<0.001	-0.75	0.634
TAPSE(mm)	24.51±3.99	23.64±4.22	22.99±4.11	0.0027	-0.87	0.036	-1.63	<0.001	-0.76	0.169
MAPSE(mm)	14.67±2.02	13.25±2.07	13.19±2.27	<0.001	-1.42	<0.001	-1.56	<0.001	-0.15	1
S'(mm)	9.54±2.04	9.08±2.06	8.97±2.18	0.0017	-0.48	0.006	-0.51	0.010	-0.04	1
LVOT VTI(cm)	21.82±4.14	19.54±3.65	19.30±4.08	<0.001	-2.38	<0.001	-2.58	<0.001	-0.2	1
SV(ml)	59.43±15.76	49.71±13.90	45.96±13.67	<0.001	-9.77	<0.001	-13.2	<0.001	-3.43	0.017
CO(ml/min)	4.74±1.32	4.08±1.17	3.60±1.08	<0.001	-0.69	<0.001	-1.07	<0.001	-0.38	0.001
<b>Longitudinal Strain</b>										
GLS(%)	-20.90±2.34	-19.60±2.83	-18.75±4.45	<0.001	-1.32	<0.001	-1.82	<0.001	-0.51	0.102
LAX(%)	-20.58±3.11	-19.44±3.40	-18.75±3.74	<0.001	-1.17	<0.001	-1.79	<0.001	-0.62	0.175
A4C(%)	-20.48±2.90	-19.29±3.10	-18.50±4.70	<0.001	-1.2	<0.001	-1.86	<0.001	-0.65	0.177
A2C(%)	-21.62±2.99	-20.01±3.10	-19.46±3.72	<0.001	-1.6	<0.001	-2.09	<0.001	-0.49	0.266
Base(%)	-17.05±2.68	-15.71±2.95	-15.57±3.36	<0.001	-1.36	<0.001	-1.64	<0.001	-0.28	0.838
Mid(%)	-20.11±2.39	-18.94±2.87	-18.55±3.25	<0.001	-1.19	<0.001	-1.55	<0.001	-0.36	0.39
Apex(%)	-25.57±3.80	-24.35±4.27	-23.17±4.41	<0.001	-1.24	<0.001	-2.05	<0.001	-0.8	0.092

**Table 5. 1:** Patients' laboratory, ECG and Echo data at each follow up visit

Hb: Hemoglobin; PR:PR interval; QTc: Corrected QT interval; LVEDV: Left ventricle end diastolic volume; LVEF: Left ventricle ejection fraction; TAPSE: Tricuspid annular plane systolic exertion; MAPSE: Mitral annular plane systolic exertion; S': Tissue Doppler peak systolic mitral annular velocity; LVOT VTI: Left ventricle out flow tract velocity time integral; SV: Stroke volume; CO: Cardiac output; GLS: Global longitudinal strain; LAX: Apical long axis; A4C: Apical 4 chamber; A2C: Apical 2 chamber. \*: p value<0.016 is considered significant.

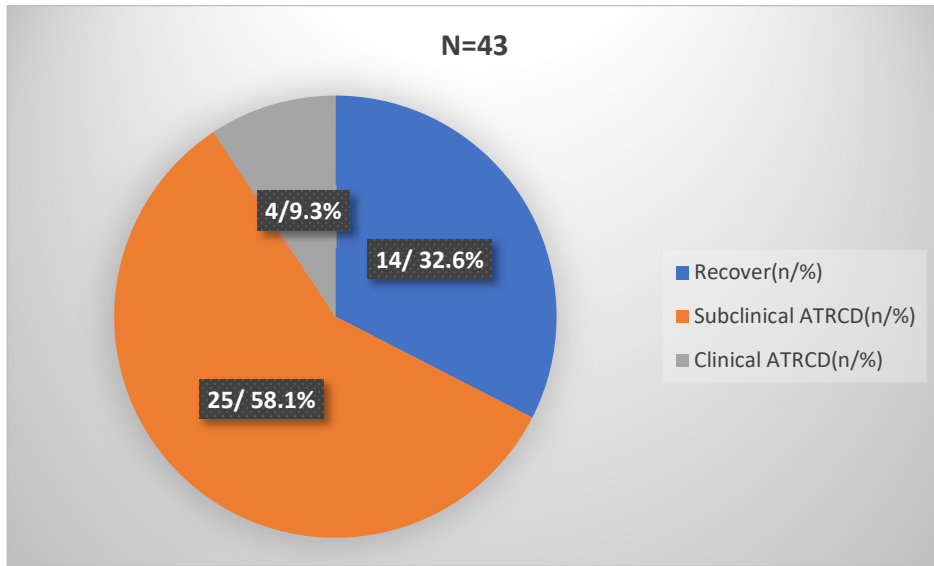
#### 5.4.2 Cumulative Incidence of ATRCD

The cumulative incidence of subclinical and clinical ATRCD at the end of anthracycline therapy were 25.1% (n=52) and 2.9% (n=6) respectively. The cumulative incidence of subclinical and clinical ATRCD at 6 months after ending anthracycline therapy were 35.0% (n=69) and 8.8% (n=16) respectively. Majority of the patients (75.3%) with subclinical ATRCD were diagnosed at the end of anthracycline therapy. Most of the patients (62.5%) with clinical ATRCD were diagnosed at 6 months after ending of anthracycline therapy. All of the patients with subclinical ATRCD were free of heart failure symptoms. Only 3 of the 16 clinical ATRCD patients had heart failure symptoms, including effort intolerance, difficulty in breathing and lower limbs oedema. One of the symptomatic patients required hospitalization due to acute decompensated heart failure with New York Heart Association functional classification IV.

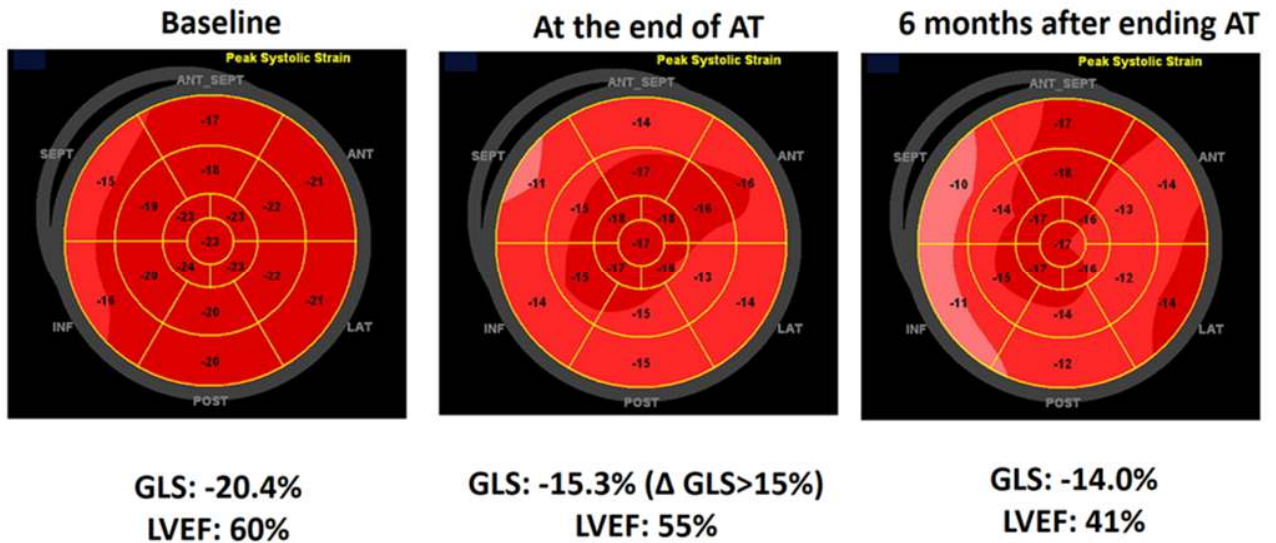
Most patients (56, 81.2%) with subclinical ATRCD were detected by GLS alone, 8 (14.3%) patients were diagnosed by positive Troponin I alone and 5 (7.2%) patients were diagnosed with subclinical ATRCD by both GLS and Troponin-I criteria.

Among the 52 patients, who were diagnosed with subclinical ATRCD at the 2<sup>nd</sup> follow-up visit (end of the anthracycline therapy), 43 patients were able to come back for the 3<sup>rd</sup> follow-up visit (6 months after ending anthracycline therapy). At the 3<sup>rd</sup> follow-up visit, 14/43 (32.6%) patients had full recovery of the GLS and/or Troponin-I, 25/43 (58.1%) patients still had subclinical ATRCD and 4/43 (9.3%) patients progressed to clinical ATRCD (**Figure 5.1**).

**Figure 5.2.** shows the strain analysis for a case who received anthracycline. At the end of anthracycline therapy, patient was diagnosed with subclinical ATRCD. At the 6 months after ending anthracycline therapy follow up, patient developed clinical ATRCD.



**Figure 5. 1:** Six months' outcome of patients who were diagnosed with subclinical ATRCD at the end of anthracycline therapy.



**Figure 5. 2:** Strain analysis for a case who received anthracycline. Bull's eye diagram demonstrates the global and regional longitudinal strain.

### 5.4.3 Predictors of ATRCD

**Table 5.2** shows the comparison of the variables among the three groups: patients who completed three study visits and found had normal cardiac function (no ATRCD, Group 0), patients who were diagnosed with subclinical (Group 1) and clinical ATRCD (Group 2) during follow-up visits. Following post-hoc analysis, patients who were diagnosed with clinical ATRCD had significantly higher proportion of HIV positive, lower baseline LVEF and lower baseline GLS ( $p<0.016$ ) than group 0 and group 1. There were no significant difference regarding age, sex, physical examination, hypertension and other cardiovascular risk factors, cancer type, stage, cumulative dose of anthracycline, chest radiation among the 3 groups.

All the 7 HIV positive patients who developed clinical ATRCD were female with the diagnosis of Breast Cancer.

On univariable analysis for the predictors of subclinical ATRCD, female sex, alcohol taking and breast cancer were found nearly not statistically significant ( $p\sim 0.10$ ). However, no factor was found to predict subclinical ATRCD in multivariable mode (**Table 5.3**). On univariable analysis, we found the development of clinical ATRCD was associated with HIV positive status (HR=3.04; 95% CI, 1.26-7.32;  $p=0.013$ ), lower baseline GLS (HR=0.61; 95% CI, 0.53-0.71;  $p<0.001$ ), lower baseline MAPSE (HR=0.72; 95% CI, 0.56-0.92;  $p=0.009$ ), lower baseline S' (HR=0.72; 95% CI, 0.56-0.92;  $p=0.009$ ), lower baseline LVEF (HR=0.78; 95% CI, 0.74-0.82;  $p<0.001$ ) and development of subclinical ATRCD at the end of anthracycline therapy (HR=6.61; 95% CI, 2.60- 16.82);  $p< 0.001$ ) (**Table 5.3**). Due to a relatively small number of clinical ATRCD ( $n=16$ ), the statistical power was reduced. Therefore, multivariable analysis was not done for the predictors of clinical ATRCD.

Variables	Group 0:#	Group 1:	Group 2:	p-value	Post-hoc p-value**		
	No ATRDC(n=82)	Subclinical ATRCD(n=65)	Clinical ATRCD(n=16)		0 vs1	0 vs 2	1 vs 2
Age, mean±SD	41.9±12.0	41.6±11.8	38.3±8.9	0.5199			
Female, n (%)	68 (82.9%)	60 (92.3%)	15 (93.8%)	0.186			
<b>Physical Examination</b>							
BMI (kg/m <sup>2</sup> ), mean±SD	25.4±4.1	25.0±4.9	26.7±5.4	0.4608			
HR, mean±SD	77.8±14.5	80.2±15.8	78.4±12.9	0.7867			
SBP (mmHg), mean±SD	127±18	129.3±17.3	135.1±19.9	0.3464			
DBP (mmHg), mean±SD	76±10	76.4±11.8	81.8±16.9	0.1778			
SaO <sub>2</sub> (%), mean±SD	97.4±2.2	97.9±1.6	97.6±1.5	0.3718			
<b>ECOG Performance Status</b>							
0, n(%)	76 (92.7%)	63 (96.9%)	16 (100%)				
1, n(%)	3 (3.7%)	2 (3.1%)	0 (0%)				
2, n(%)	3 (3.7%)	0 (0%)	0 (0%)	0.585			
<b>Cardiovascular Risk Factors</b>							
Hypertension, n(%)	12 (14.6%)	13 (20.0%)	4 (2.6%)	0.468			
DM, n(%)	2 (2.4%)	2 (3.1%)	0 (0%)	1			
CKD, n(%)	1 (1.2%)	0 (0%)	0 (0%)	1			
HIV Positive, n(%)	12 (14.6%)	9 (13.9%)	7 (43.5%)	<b>0.012</b>	0.892	<b>0.007</b>	<b>0.005</b>
Obesity, n(%)	9 (10.9%)	9 (13.9%)	4 (25%)	0.322			
Alcohol use, n (%)	16 (19.5%)	8 (12.3%)	3 (18.8%)	1			
Smoking, n (%)	1 (1.2%)	0 (0%)	0 (0%)	1			
<b>Cancer Profile</b>							
Breast cancer, n (%)	63 (76.8%)	56 (86.2%)	14 (87.5%)				
Non-Hodgkin's lymphoma, n(%)	5 (6.1%)	5 (7.7%)	0 (0%)				
Hodgkin's lymphoma, n (%)	7 (8.5%)	3 (4.6%)	0				
Sarcomas, n (%)	7 (8.5%)	1 (1.5%)	2 (12.5%)	0.256			
Stage 1, n (%)	10 (12.5%)	11 (18.3%)	1 (6.25%)				
Stage 2, n (%)	20 (25.0%)	13 (21.7%)	1 (6.25%)				
Stage 3, n (%)	33 (41.3%)	28 (46.7%)	9 (56.3%)				

Stage 4, n (%)	17 (21.3%)	8 (13.3%)	5 (31.2%)	0.325			
Cumulative dose of anthracycline,mg/m2 (mean±SD)	299.3±6.3	315.3±50.8	323.4±45.8	0.575			
Left chest radiation, n (%)	19 (27.1%)	10 (21.2%)	2 (13.3%)	0.51			
Right chest radiation, n (%)	11 (17.7%)	13 (26.0%)	1 (7.1%)	0.295			
Taking cardioprotective drugs,* n (%)	1 (1.2%)	3 (4.6%)	1 (6.3%)	0.284			
<b>Baseline Lab</b>							
Hb (g/dL), mean±SD	12.9±1.5	12.7±2.7	13.5±1.5	2.2653			
eGFR (ml/min/1.73m <sup>2</sup> ), mean±SD	104.3±29.3	98.4±32.2	119.3±28.8	0.06			
TnI (ng/ml)	0.09±0.19	0.96±0.07	0.82±0.05	0.3718			
<b>Baseline ECG</b>							
PR(ms), mean±SD	104.3±29.3	98.4±32.2	119.3±28.8	0.06			
QTc(ms), mean±SD	411.2±35.3	409.1±23.8	401.6±31.4	0.5216			
<b>Baseline ECHO</b>							
LVEF(%) ,mean±SD	60.9±5.2	62.3±6.1	54.6±2.9	<0.001	0.353	<0.001	<0.001
MAPSE(mm), mean±SD	14.8±2.2	14.8±1.9	13.5±1.8	0.06			
S'(m/s), mean±SD	9.6±1.9	9.6±2.1	8.6±1.5	0.2024			
Impaired relaxation,n(%)	26 (31.7%)	17 (31.7%)	5 (31.3%)	0.754			
<b>Baseline Strain</b>							
GLS(%)	20.4±2.1	22.1±2.3	18.7±1.31	<0.001	0.083	0.016	<0.001

**Table 5. 2:** Patients characteristic at the baseline and cancer profile in 3 different groups.

BMI: Body mass index, HR: Heart rate, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, SaO<sup>2</sup>: Oxygen saturation, NHL: non-Hodgkin's lymphoma, HL: Hodgkin's lymphoma. Hb: Hemoglobin; PR:PR interval; QTc: Corrected QT interval; LVEF: Left ventricle ejection fraction; MAPSE: Mitral annular plane systolic exertion; S': Tissue Doppler peak systolic mitral annular velocity; GLS: Global longitudinal strain.

\*: taking one or more cardioprotective drugs, including: Bata blockers, Angiotensin-converting enzyme inhibitors, Angiotensin II receptor blockers

\*\* : *p* value obtained by 2x2 comparisons with Bonferroni correction

#: Group 0 included patients who completed 3 follow up visits and were found to have no evidence of cardiotoxicity during follow up visits. Patients who didn't come back for the 3<sup>rd</sup> visit were exclude from Group 0.

Predictor	Subclinical ATRCD				Clinical ATRCD	
	Univariable models		Multivariable model		Univariable models	
	Hazard Ratio (95% CI)	<i>p</i> - value	Hazard Ratio (95% CI)	<i>p</i> - value	Hazard Ratio (95% CI)	<i>p</i> - value
Female	2.10 ( 0.89, 4.95)	<b>0.09</b>	1.73 (0.64, 4.71)	0.28	2.36 (0.31, 18.00)	0.408
Age, years	0.99 (0.98, 1.01)	0.607	0.98 (0.96, 1.01)	0.071	0.98 (0.94, 1.01)	0.162
Systolic BP, mmHg	0.99 (0.98, 1.01)	0.382			1.01 (0.99, 1.01)	0.189
Diastolic BP, mmHg	0.99 (0.97, 1.01)	0.342			1.04 (0.98, 1.01)	0.223
Hypertension	0.99 (0.62, 1.62)	0.994			1.57 (0.56, 4.37)	0.388
HIV positive	0.92 (0.52, 1.60)	0.757			3.04 (1.26, 7.32)	<b>0.013</b>
BMI	0.98 (0.94, 1.03)	0.527			1.06 (0.96, 1.18)	0.246
Taking alcohol	0.56 (0.29, 1.12)	<b>0.102</b>	0.60 (0.31, 1.16)	0.126	1.06 (0.30, 3.73)	0.326
Hemoglobin, g/dl	0.98 (0.87, 1.11)	0.789			1.28 (0.99, 1.64)	0.055
eGRF	0.99 (0.99, 1.00)	0.248			1.02 (0.99, 1.03)	0.24
Breast Cancer	1.82 (0.95, 3.49)	<b>0.073</b>	1.13 (0.48, 2.61)	0.784	1.86 (0.43, 8.07)	0.405
Stage 3/4 disease	0.76 (0.49,1.19)	0.23			3.75 (0.88, 15.96)	0.074
Cumulative AT dose, mg/m2	1.00 (0.99,1.01)	0.138			1.01 (1.00, 1.02)	0.234
Baseline GLS	1.01(0.98, 1.36)	0.769			0.61 (0.53, 0.71)	<b>&lt;0.001</b>
Baseline MAPSE	1.01(0.91, 1.11)	0.918			0.72 (0.56, 0.92)	<b>0.009</b>
Baseline S'	0.99 (0.89, 1.10)	0.886			0.74 (0.57, 0.97)	<b>0.030</b>
Impaired diastolic dysfunction	0.92 (0.57, 1.47)	0.722			1.11 (0.40, 3.12)	0.842
Baseline LVEF	1.03 (0.99, 1.07)	<b>0.051</b>	1.05 (0.89, 1.09)	0.086	0.78 (0.74, 0.82)	<b>&lt;0.001</b>
Subclinical ATRCD at end of therapy					6.61 (2.60, 16.82)	<b>&lt;0.001</b>

**Table 5. 3:** Predictor of developing ATRCD

BP: Blood pressure, BMI: Body mass index, eGFR: Estimated glomerular filtration rate, LVEF: Left ventricle ejection fraction; MAPSE: Mitral annular plane systolic exertion; S': Tissue Doppler peak systolic mitral annular velocity; GLS: Global longitudinal strain.

#### **5.4.4 Sub analysis of the patients who lost to follow up**

There were 62 patients who didn't come back for the 3<sup>rd</sup> visit after 6 month of completing anthracycline therapy, including 27 patients died of cancer, 16 patients were last know alive and declined to come due to challenges rising from Covid-19 pandemic and 19 patients who were failed to be reached by phone and were considered as lost to follow up. **Table 5.4** summarizes the data of this group of patients. Results of independent t-test (for Gaussian distributed data) and Mann-Whitney (for Gaussian distributed data) were also demonstrated. There was no significant difference between this group and the patients who completed the study protocols, regarding the baseline demographics, cancer profile, physical examinations and investigations at baseline and 2<sup>nd</sup> visit (end of anthracycline therapy).

<b>Variables</b>	<b>Completed F/U visit (n=145)</b>	<b>Missed 3<sup>rd</sup> visit (n=62)</b>	<b>p-value</b>
Age, mean±SD	41.5±11.9	43.2±11.8	0.156
Female, n (%)	124 (85.7%)	53 (86.3%)	0.903
<b>Physical Examination</b>			
BMI (kg/m <sup>2</sup> ), mean±SD	25.2±4.4	25.8±5.4	0.204
HR, mean±SD	77.3±14.1	79.9±15.6	0.172
SBP (mmHg), mean±SD	128.1±16.8	130.2±17.7	0.418
DBP (mmHg), mean±SD	75.8 10.8	77.3 12.4	0.512
SaO <sub>2</sub> (%), mean±SD	97.8±1.7	97.3±2.2	0.195*
<b>Cardiovascular Risk Factors</b>			
Hypertension, n (%)	36 (25.2%)	17 (27.4%)	0.412
DM, n (%)	1 (0.03%)	0	0.150
CKD, n (%)	1 (0.03%)	0	0.150
HIV Positive, n (%)	26 (18.0%)	14 (22.9%)	0.511
Obesity, n (%)	17 (11.5%)	8 (12.3%)	0.531
Alcohol use, n (%)	25 (17.3%)	12 (20.0%)	0.629
Smoking, n (%)	1 (0.03%)	0	0.150
<b>Cancer Profile</b>			
Breast Cancer, n (%)	117 (81.3%)	46 (74.3%)	
Non-Hodgkin's lymphoma, n (%)	8 (5.8%)	6 (10.0%)	
Hodgkin's lymphoma, n (%)	10 (7.2%)	4 (7.1%)	
Sarcomas, n (%)	7 (5.0%)	4 (7.1%)	0.506
Stage 1, n (%)	23 (16.0%)	6 (9.8%)	
Stage 2, n (%)	31 (21.4%)	9 (14.8%)	
Stage 3, n (%)	65 (45.0%)	35 (55.7%)	
Stage 4, n (%)	25 (17.6%)	12 (19.7%)	0.366
Cumulative dose of anthracycline (mg/m <sup>2</sup> ), mean±SD	309.6±56.5	312.6±53.5%	0.851
<b>Baseline investigations</b>			
Hb (g/dl), mean±SD	12.8±1.8	12.7±2.1	0.852
eGFR (ml/min/1.73m <sup>2</sup> ), mean±SD	103.8±29.5	100.1±2.9	0.784
TnI (ng/ml), mean±SD	0.1±0.1	0.1±0.1	0.833*
PR (ms), mean±SD	151.2±21.8	156±22.0	0.241
QTc (ms), mean±SD	407.7±24.9	414.8±36.7	0.241
LVEF (%) ,mean±SD	60.7±5.8	61.3±5.8	0.233
MAPSE (mm), mean±SD	14.7±2.1	14.6±1.9	0.583
S' (m/s), mean±SD	9.4±1.8	9.8±2.3	0.553*
GLS (%), mean±SD	-20.9±2.3	-21.0±2.4	0.617
<b>2nd visit investigations</b>			
Hb (g/dl), mean±SD	11.9±1.6	12.1±1.9	0.303
eGFR (ml/min/1.73m <sup>2</sup> ), mean±SD	105.5±35.8	100.5±33.1	0.601
TnI (ng/ml), mean±SD	0.1±0.1	0.1±0.2	0.263*

PR (ms), mean±SD	152.4±21.6	153.4±18.5	0.372
QTc (ms), mean±SD	415.3±23.2	399.2±57.6	0.067
LVEF (%) ,mean±SD	57.4±6.0	57.9±6.5	0.617
MAPSE (mm), mean±SD	13.4±2.0	13.4±2.1	0.567
S' (m/s), mean±SD	9.0±2.0	9.3±2.2	0.381*
GLS (%), mean±SD	-19.5±2.7	-19.8±3.1	0.411
<b>2nd visit diagnosis</b>			
No ATRCD, n (%)	108 (74.5%)	43 (69.1%)	
Subclinical ATRCD, n (%)	34 (23.4%)	17 (27.9%)	
Clinical ATRCD, n (%)	3 (2.2%)	2 (2.9%)	0.777

**Table 5. 4** Characteristics of the patients who missed 3<sup>rd</sup> visit

BP: Blood pressure, BMI: Body mass index, eGFR: Estimated glomerular filtration rate, LVEF: Left ventricle ejection fraction; MAPSE: Mitral annular plane systolic exertion; S': Tissue Doppler peak systolic mitral annular velocity; GLS: Global longitudinal strain. F/P: Follow up; ATRCD: Anthracycline therapy related cardiac dysfunction

\*: *p* value calculated using Mann-Whitney test

## 5.5 Discussion

### 5.5.1 Cumulative Incidence of subclinical ATRCD

In this cancer cohort which underwent anthracycline based chemotherapy, for the first time in our setting, we implemented international cardiac surveillance recommendation. Patients were followed up to 6 months after ending anthracycline therapy, using LVEF, GLS and troponin I as the tool of cardiac monitoring. We found a high cumulative incidence of subclinical ATRCD of 35.0% at 6 months after ending anthracycline therapy. The result is different from similar studies. Only few publications reported the incidence of subclinical ATRCD. Boyd, A. group (Boyd et al., 2017) used two dimensional (2D) strain analysis, to detect early subclinical LV systolic dysfunction in 140 breast cancer patients within three month after anthracycline chemotherapy. Subclinical LV dysfunction (>11% reduction in GLS) occurred in 22% of their patient cohort. In another cohort of 159 patients receiving anthracycline, trastuzumab (a monoclonal antibody for

treating HER2 receptor positive breast cancer), or both (Negishi et al., 2014), decreased GLS (by >11%) was found in 33% patients 7 months after the completion of the chemotherapy treatment. Both studies (Boyd et al., 2017, Negishi et al., 2014) diagnosed subclinical ATRCD by GLS alone, without troponin. The different chemotherapy (anthracycline + trastuzumab vs anthracycline) and diagnostic protocols (GLS alone vs GLS + troponin) and cut-off of GLS value (decrease > 11% vs  $\geq 15\%$ ) have contributed to different results among these studies and present research.

Detection of subclinical ATRCD was made mainly by GLS alone (81.2%) in the present study. Only few subclinical cases fulfilled troponin criteria alone (14.3%), which is inconsistent with other registries (Pareek et al., 2018). Pareek and colleagues reported 59% of the subclinical cardiotoxicity cases were defined by biomarker criteria (raised TnI or brain natriuretic peptide). This disparity is due to the troponin test used in our study was not an ultra-sensitive TnI test with a cutoff point of 0.3ng/ml, therefore has relatively lower sensitivity to detect the cardiotoxicity than the ultra-sensitive TnI test was used by Pareek group with a cutoff point of 0.02ng/ml.

### **5.5.2 Cumulative Incidence of ATRCD**

The cumulative incidence of clinical ATRCD at 6 months ending of anthracycline therapy was 8.8%. This finding is similar with the result reported in the biggest cancer cohort by Cardinale and colleagues (Cardinale D, 2015), who showed that the overall incidence of cardiotoxicity was 9% with the median follow-up of 5.2 years. They found 98% cases were diagnosed within the first year following the ending anthracycline therapy. Given that patients in the present study were followed up to 6 months after anthracycline therapy, the one-year cumulative incidence of clinical ATRCD could be higher in our study population than in Cardinale's study population.

### **5.5.3 Time course of detecting subclinical and clinical ATRCD**

Most of the patients (75.3%) with subclinical ATRCD were detected early (at the end of anthracycline therapy). In contrast, most of the patients (62.5%) with clinical ATRCD were diagnosed relatively late (at 6 months after ending of anthracycline therapy) and 4 patients of the clinical ATRCD patients, who were diagnosed at 3<sup>rd</sup> visit, progressed from subclinical disease detected during the 2<sup>nd</sup> visit. These observations underline that GLS reduction precedes LV dysfunction in patients who later develop heart failure (Lambert et al., 2020). Strain abnormalities can be seen early despite preserved LVEF, therefore can be used as a tool to detect subclinical ATRCD.

### **5.5.4 Clinical presentations of subclinical and clinical ATRCD**

Consistent with other registries (Pareek et al., 2018, Cardinale D, 2015), all the patients who had subclinical ATRCD were asymptomatic for heart failure and the rate of asymptomatic clinical ATRCD was high. In a large cancer cohort of Cardinale group, cardiotoxicity occurred in 226 (9%) patients. One hundred eighty-three (81%) patients, were in New York Heart Association class I to II, and 43 (19%) were in class III to IV. HF therapy (enalapril, carvedilol/ bisoprolol) was initiated in all patients developing cardio-toxicity. One hundred eighty-five (82%) patients recovered from cardiotoxicity with 25 (11%) had full recovery, and 160 (71%) had partial recovery. Patients who did not recover had a higher New York Heart Association class and were less likely to tolerate the association of enalapril and  $\beta$ -blockers. Notably, patients who did not recover from cardiotoxicity had a higher incidence of adverse cardiac events (Cardinale D, 2015). This reflects the periodic cardiac monitoring leading to early detecting and management of cardiotoxicity with easily available and affordable drugs, therefore a better outcome.

At the end of follow-up, a lower LVEF and GLS than baseline was also observed in patients who did not develop subclinical or clinical ATRCD, the same trends have been observed in other studies (Mornoş and Petrescu, 2013, Negishi et al., 2014, Baratta S., 2013, Sawaya et al., 2012). This finding may imply there is still significant decline in myocardial contractility in patients who didn't fulfil the criteria of ATRCD after anthracycline exposure. A longer follow up period is needed for studying the long term trend of myocardial function after anthracycline exposure.

#### **5.5.5 Predictors of subclinical and clinical ATRCD**

We identified some predictors of clinical ATRCD in univariable analysis including, HIV positive, lower baseline GLS and LVEF, diagnosis of subclinical ATRCD at the end of anthracycline therapy. Although the relationship between subclinical ATRCD and later development of clinical ATRCD risk is well recognized (Sawaya et al., 2011, Fallah-Rad et al., 2011, Stoodley et al., 2011, Mavinkurve-Groothuis et al., 2013), the association between HIV, baseline GLS, LVEF and cardiotoxicity development in adult patients have never been reported, thus far.

Researches done in pre and post-ART era showed people with HIV has higher risk of heart failure than the general population even after excluding the traditional cardiovascular risk factors, especially those who were younger than 40, female (Go et al., 2022, Al-Kindi et al., 2016, Feinstein et al., 2018, Freiberg et al., 2017, Womack et al., 2014).

There is increasing evidence that persistent immune dysregulation, despite early initiation of ART, plays an important role in the development of myocardial dysfunction either directly or through enhancing the effects on traditional cardiovascular risk factors (Sinha and Feinstein, 2020). In a cross sectional imaging study, treated HIV patients without underlying cardiovascular disease had increased levels of intramyocardial lipid and fibrosis compared to matched uninfected controls on

cardiac magnetic resonance imaging. These findings strongly correlated with increased levels of markers associated with inflammation and monocyte activation (Thiara et al., 2015). Similarly, women with HIV on ART had increased myocardial fibrosis compared to matched uninfected controls. The myocardial fibrosis significantly correlated with markers of monocyte activation (Zanni et al., 2020).

HIV related immune dysregulation has also been proposed to be one of the mechanism of fostering cancer, especially non-AIDS-defining cancer (Casper C, 2017). AIDS-Cancer registries conducted in Uganda (Mbulaiteye et al., 2006), Nigeria (Akarolo-Anthony et al., 2014) and South Africa (Sengayi et al., 2016) confirmed the increased risks of both AIDS-defining cancers and non-AIDS-defining cancers among the people with HIV infection.

In the present study, cancer patients with HIV were found to have higher risk of developing ATRCD. All the seven patients with HIV and ATRCD were female patients with breast cancer. If HIV is a shared risk factor for heart failure and cancer through immune dysregulation, then the response of immune cells to the cardiac insult-anthracycline is important in understanding the subsequent development of ATRCD.

There is a strong need for further delineating the pathways that lead from immune dysregulation to myocardial dysfunction in anthracycline treated HIV cancer patients. Our findings also bring particular attention of the joint care of the oncologists, cardiologists and infectious disease specialist on HIV cancer patients who need anthracycline or other cardio toxic chemotherapy.

We didn't find any associations between baseline cardiovascular risk factors and the development of clinical disease as other researchers' reported (Cai et al., 2019, Qiu et al., 2021, Armstrong et al., 2013). However, we had previously found obesity was one of the most prevalent (13%)

cardiovascular risk factors and associated with suboptimal (lower) baseline GLS ( absolute  $GLS \leq 18\%$ ) in the same cancer population (Zhang et al., 2021). Given that present study showed the association between lower baseline GLS and the development of clinical ATRCD. We can still infer obesity could be a predictor of ATRCD. In our study, even if the cumulative anthracycline dose appeared the highest in clinical ATRCD group and lowest in no cardiotoxicity group, it didn't turn out to be a predictor of subclinical or clinical ATRCD. Elsewhere, ATRCD has been reported as dose dependent (Blanco et al., 2012). A larger multicenter study with a longer follow up period may be needed to further study the predictors of subclinical ATRCD and clinical ATRCD, particularly on cardiovascular risk factors and anthracycline doses in this population.

## **5.6 Limitations**

At present, our study is the biggest anthracycline therapy cohort in Uganda. However, it has several limitations. First, being a single center study, we included a population admitted to a single center. However, this center receives patients from all over Uganda and therefore there is some national representation among population studied. Second, the high prevalence of women, driven by a high proportion of breast cancer patients in our study population, would limit the generalizability of our findings to the entire cancer population. Third, the Covid-19 pandemic posted a big challenge on patients' follow-up visits. Seventeen (8.2%) patients were not able or not willing to come back to the study site for follow-up visit due to travel restrictions and increased cost of travelling. Forth, the ECHO observers were not blinded to patients' status - this could have led to potential measurement bias and misclassification. However, automatic software was used for the strain analysis and 2D LVEF measurement, which we think could have minimize the bias. Fifth, due to the relatively small number of clinical ATRCD events, the statistical power was reduced, therefore a multivariable analysis to identify the independent

predictors for clinical ATRCD was not performed. However, the one-way ANOVA test and univariable analysis can still shed light on that.

### **5.7 Conclusion and future perspective**

There is high cumulative incidence of ATRCD among Uganda cancer patients. Most of the patients who had ATRCD were detected at subclinical stage or asymptomatic clinical stage.

Development of clinical ATRCD is associated with HIV positive, low baseline GLS or LVEF and diagnosis of subclinical ATRCD at the end of anthracycline therapy. Therefore, cardiac surveillance at baseline and ending of anthracycline therapy are essential to identify high risk patients of developing clinical ATRCD in the future, particularly in HIV positive cancer patient.

This can guide the establishment of local cardio-oncology guideline in patients receiving anthracycline therapy and early start of pharmacological therapy could be considered.

This research will draw a special attention to the cardiologists and oncologists in Africa on joint care for cancer patients, lay a foundation of cardio-oncology practice in Uganda, including setting up a cardio-oncology clinic and developing local guidelines appropriate to Ugandan patients and health facilities, stimulating more researches in cardio-oncology field.

## **Chapter 6: The role of conventional ECHO to detect subclinical ATRCD**

### **6.1 Introduction**

We have discussed our findings in chapter 5 that there is a high cumulative incidence (35% at 6 months after ending of anthracycline therapy) of subclinical ATRCD in Uganda cancer patients and the diagnosis of subclinical disease predicts the development of clinical ATRCD (HR: 6.61; 95% CI:2.60, 16.82;  $p < 0.001$ ). From our data and the evidence from other researcher (Cardinale D, 2015), we believe detecting ATRCD at subclinical stage will identify the patients at high risk and leads to close monitoring, prompt therapy and better outcome.

GLS obtained by STE is currently the most accepted diagnostic tool for subclinical ATRCD (Plana et al., 2014). It is a widely used modality for cardio-oncology practice in developed world, but not the case in a low to middle income country. In Uganda, STE is not available in most of the hospital. Moreover, STE requires adequate visualization of the endocardial border and is heavily image quality dependent. This can further limit its application.

When STE technology is not available or not feasible, the quantitation of LV longitudinal function by simple conventional ultrasound tools such as MAPSE by M-mode echocardiography, or the S' by TDI, could be useful adjunct information to LVEF in the evaluation of LV systolic function (Ewer MS and SM, 2009, Karakurt et al., 2008, Ganame et al., 2007, Tassan-Mangina et al., 2006, Kapusta et al., 2000). MAPSE and S' can be easily measured using all the echocardiographic machines and are less dependent on image quality. The current international recommendation is that a progressive decline of MAPSE should raise concern for subclinical LV dysfunction (Plana et al., 2014). However, no study has defined its cutoff values that allow the prediction of ATRCD. In this chapter, we present and discuss the result of the sub analysis of the longitudinal data, aiming to investigate the correlations between GLS and MAPSE/S', evaluate the roles of MAPSE and S'

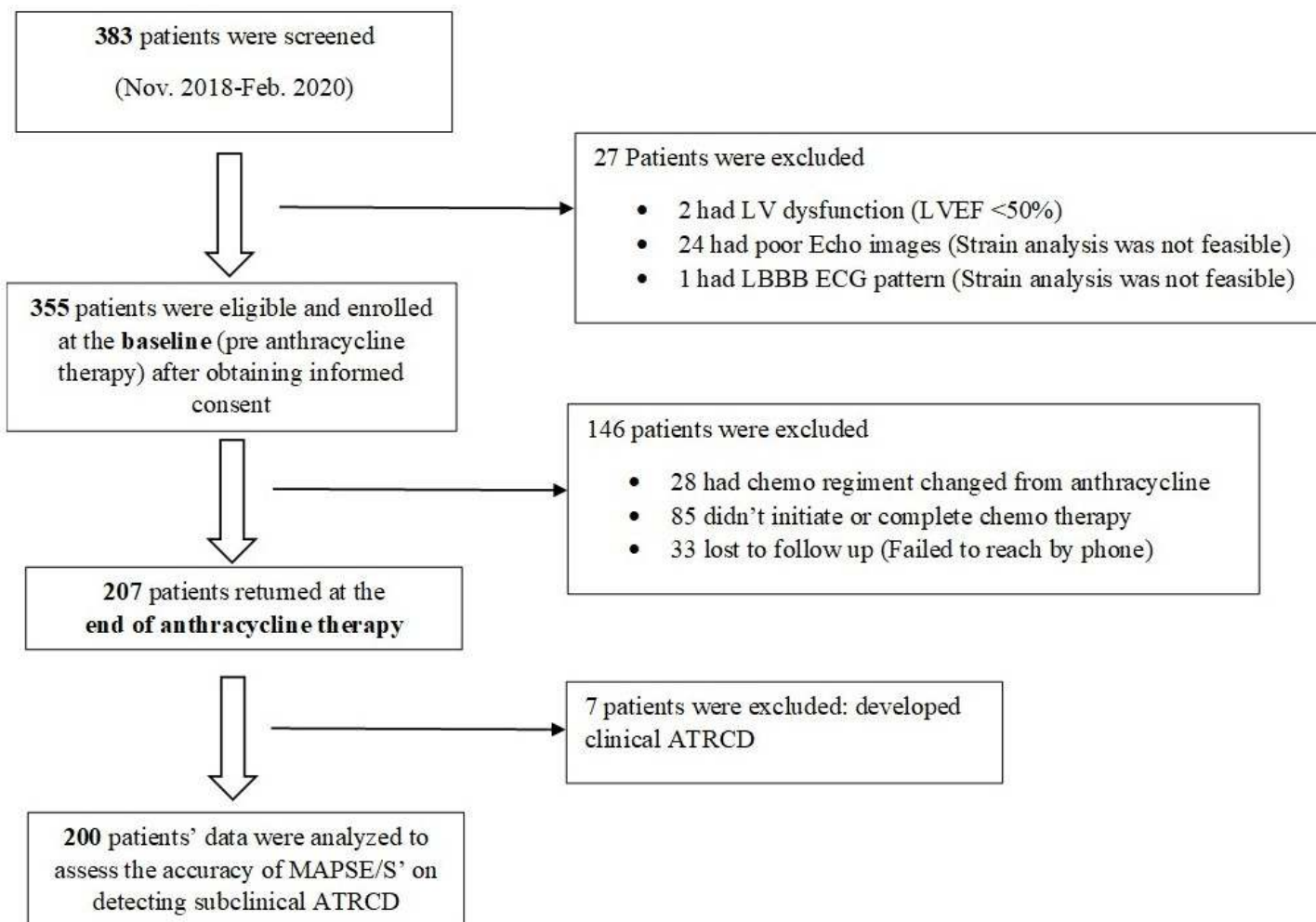
on detecting subclinical ATRCD among patients attending Uganda Cancer Institute. We hypothesized that MAPSE/S' are positively correlated with GLS and MAPSE/S' detect subclinical ATRCD with a sensitivity of  $\geq 70\%$  and a specificity  $\geq 55\%$ .

## 6.2 Method

We performed a sub analysis of the SATRACD study, which is a cancer cohort who underwent anthracycline based chemotherapy. The detailed description of the SATRACD study design, patient selection, and data collection has been presented in chapter 3.

The minimum number of participants required to investigate the accuracy of MAPSE/S' was calculated using MedCalc® Statistical Software version 20.111 (MedCalc Software Ltd, Ostend, Belgium). Where Type I error ( $\alpha$ , Significance) = 0.05; Type II error ( $\beta$ , 1-Power) = 0.20; Area under the curve = 0.7; Null Hypothesis value = 0.5; Ratio of sample sizes in negative / positive groups = 3; Then the minimum number of positive cases required = 22; The minimum number of negative cases required = 66; Total minimum sample size (both groups together) = 88.

Between November 2018 and February 2020, 355 patients were recruited at the baseline (pre-chemotherapy). Among them, 207 patients completed anthracycline therapy and paid for the 2<sup>nd</sup> visit at the end of anthracycline therapy. At the baseline, patient's demographics, cancer profile and past medical history were recorded. Patients' symptoms, physical examinations, ECG, echocardiographic and laboratory data were collected at baseline and end of anthracycline therapy. Baseline's and 2<sup>nd</sup> visit's data of the 200 patients who had no evidence of clinical ATRCD at the end of anthracycline therapy was extracted and analyzed (**Figure 6.1**).



**Figure 6. 1:** Patients' flow chart for conventional ECHO assessment

The Definition of subclinical ATRCD and clinical ATRCD, Echocardiography protocol and equipment are presented in chapter 4 and 5. To examine inter and intra-observer reliability, we reassessed LV GLS, MAPSE and S' assessments in a random sample of 10 patients. The intraclass correlation coefficients for inter and intra-observer reliability were 0.93 and 0.91 respectively for LV GLS, 0.89 and 0.91 respectively for MAPSE, 0.90 and 0.91 respectively for S' assessments.

### 6.3 Statistical analysis

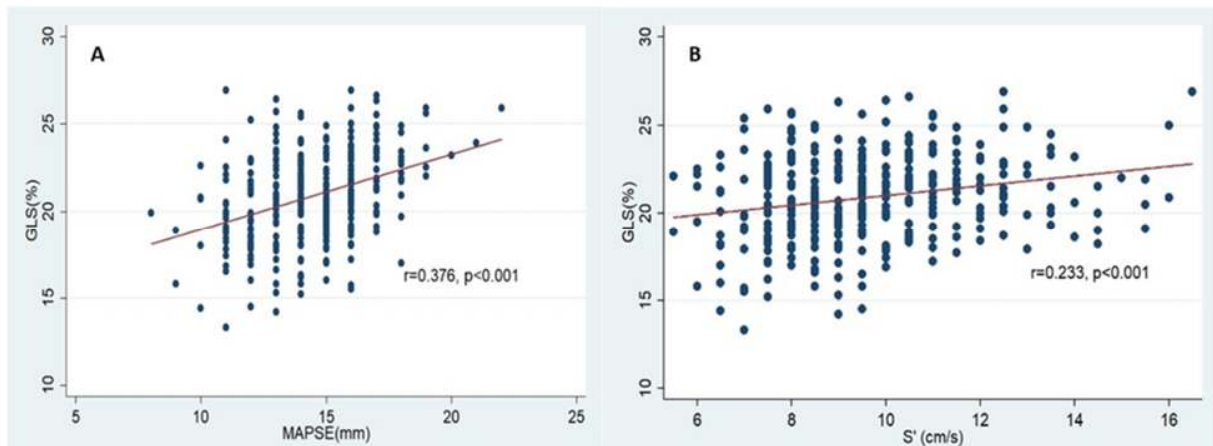
We analyzed the data using STATA v14 (Institute Inc., College Station, TX, USA). All continuous variables were expressed as a mean  $\pm$  SD or median (interquartile range) and categorical variables as frequencies and percentages. Paired t test (for Gaussian distribution) and Wilcoxon signed rank test (for non-Gaussian distribution) were used to compare dependent continuous variables, where appropriate. Pearson correlation was used to assess the correlation between GLS and MAPSE/S'. Receiver operating characteristic (ROC) analysis was used to assess the accuracy of the 2 conventional echocardiographic parameters on diagnosing subclinical ATRCD. GLS measured by STE was used as the gold standard diagnostic test to define the case. Empirical cutoff point estimation was done using nearest to (0,1) method. A two-sided *p*-value  $<0.05$  was considered statistically significant for all analyses.

### 6.4 Results

In total, 207 patients enrolled at the baseline, were able to complete anthracycline therapy and returned at the end of the anthracycline therapy. Among them, 7 patients developed clinical ATRCD and were excluded from the data analysis (**Figure 6.1**).

#### 6.4.1 Correlation between GLS and MAPSE/S'

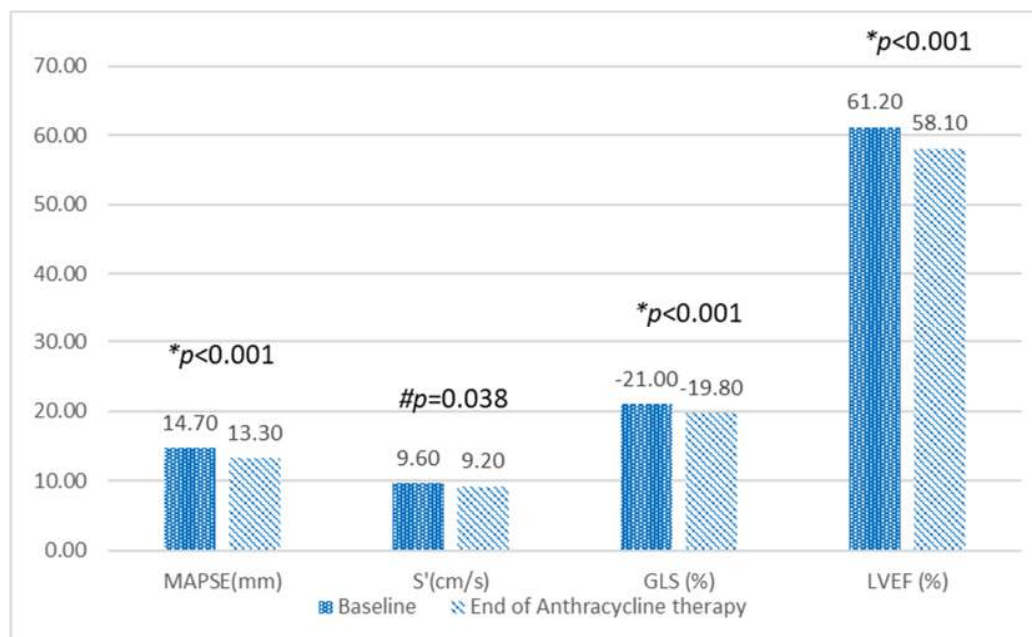
Among the 200 patients who entered data analysis, 172 (86.0%) were female, with a median (IQR) age of 42 (20-69) years. Both conventional echocardiographic parameters- MAPSE and S', showed significant positive correlations with the absolute GLS at the baseline ( $r=0.376$ ,  $p<0.001$  and  $r=0.233$ ,  $p<0.001$  respectively) (**Figure 6.2**). This correlation persisted at the end of the anthracycline therapy ( $r=0.529$ ,  $p<0.001$  for MAPSE and absolute GLS;  $r=0.353$ ,  $p<0.001$  for S' and absolute GLS).



**Figure 6. 2:** Correlation of GLS and MAPSE/ $S'$  at the baseline

A: Correlation of GLS and MAPSE; B: Correlation of GLS and  $S'$

At the end of the anthracycline therapy, LVEF, GLS, MAPSE and  $S'$  showed significant reduction compared to baseline measurements (**Figure 6.3**). There were 47 (23.5%) patients who were diagnosed with subclinical ATRCD at the end of anthracycline therapy by GLS criteria.



**Figure 6. 3:** Compare echocardiographic variables at the baseline and at the end of anthracycline therapy

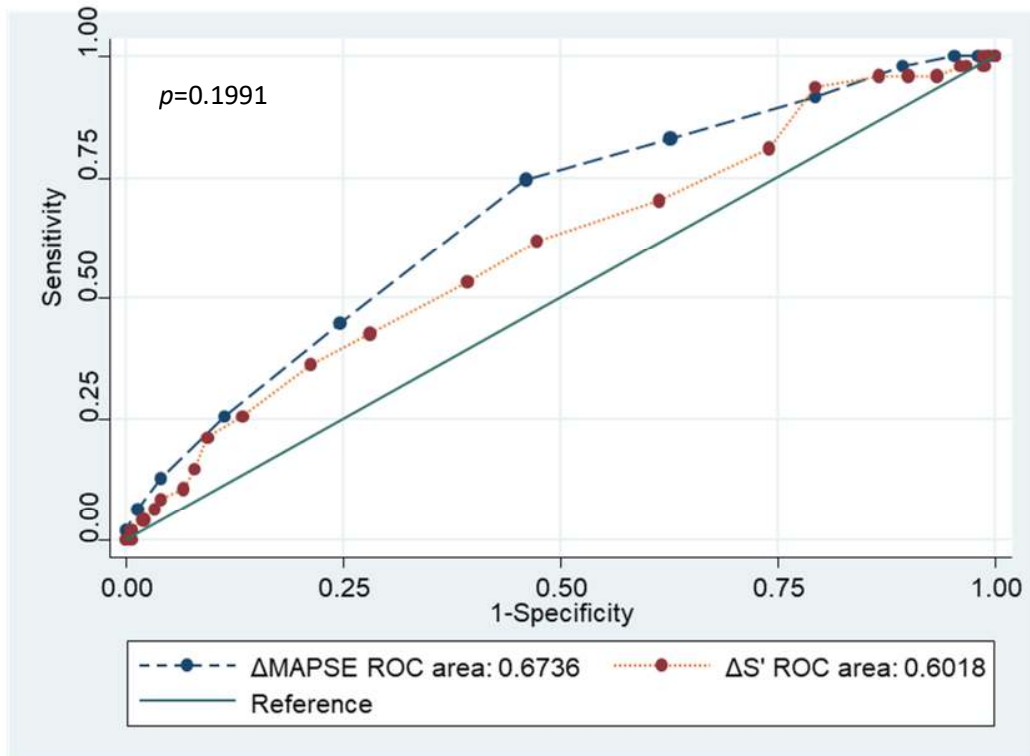
MAPSE: Mitral annular plane systolic exertion;  $S'$ : Mitral annular peak systolic tissue Doppler velocity; GLS: Global longitudinal strain; LVEF: left ventricular ejection fraction

\*: *p* value calculated using paired t test

#: *p* value calculated using Wilcoxon signed rank test

#### 6.4.2 Accuracy of MAPSE/ S' compared to GLS

**Figure 6.4** and **Table 6.1** show receiver operating characteristic analysis results of the 2 conventional echocardiographic parameters: Reduction of MAPSE ( $\Delta$ MAPSE) and reduction of S' ( $\Delta$ S'). The area under the curve (AUC), cutoff point, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of  $\Delta$ MAPSE were 0.6736 (95% CI: 0.5885, 0.7587),  $\geq 2$ mm, 74.5% (95% CI: 59.7%, 86.1%), 54.9% (95% CI: 46.7%, 63.0%), 33.7% (95% CI: 24.7%, 43.6%) and 87.5% (95% CI: 79.2%, 93.4%). The AUC, cutoff point, sensitivity, specificity, PPV and NPV of  $\Delta$ S' were 0.6018 (95% CI: 0.5084, 0.6953),  $\geq 0.5$ cm/s, 61.7% (95% CI: 46.4%, 75.5%), 52.7% (95% CI: 44.4%, 60.9%), 29.0% (95% CI: 20.4%, 38.9%) and 76.1% (95% CI: 72.4%, 88.6%). When  $\Delta$ MAPSE and  $\Delta$ S' are used as parallel test, meaning both tests are performed in each patient and subclinical ATRCD is considered when any of the test is positive, the net sensitivity and specificity is 89.4% and 28.8% respectively, the net PPV and NPV is 27.8% and 90.0% respectively.



**Figure 6. 4:** Receiver operating characteristic curve comparing the two conventional echocardiographic parameters ( $\Delta$ MAPSE and  $\Delta$ S')

ROC: Receiver operating characteristic;  $\Delta$ MAPSE: Reduction of mitral annular plane systolic exertion;  $\Delta$ S': Reduction of mitral annular peak systolic tissue Doppler velocity

\*: p-value comparing the accuracy of the 2 conventional echocardiographic parameters

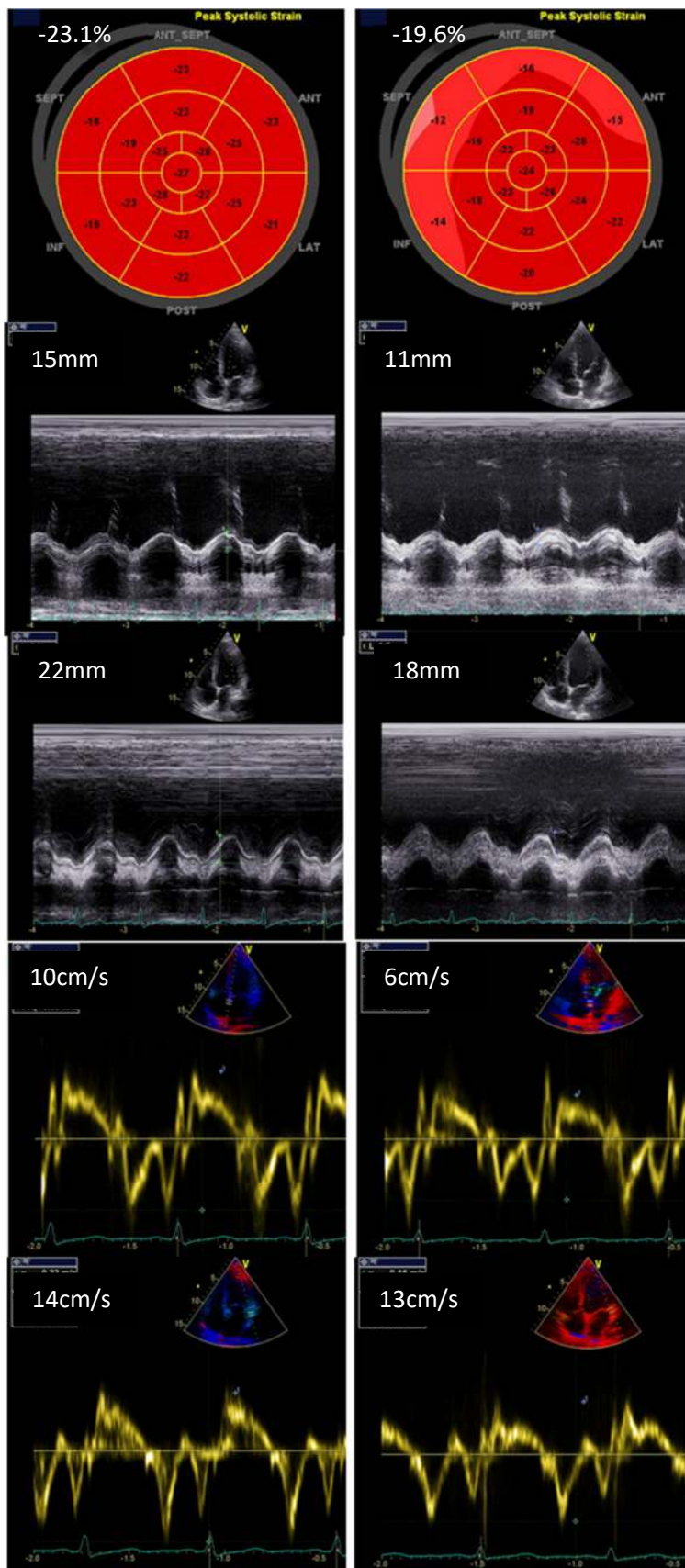
Conventional Echocardiographic parameters	Cutoff point*	Sensitivity (95% CI)	Specificity (95% CI)	AUC (95%CI)	PPV (95%CI)	NPV (95%CI)
$\Delta$ MAPSE(mm)	$\geq 2$	74.5% (59.7%, 86.1%)	54.9% (46.7%, 63.0%)	0.6736 (0.5885, 0.7587)	33.7% (24.7%, 43.6%)	87.5% (79.2%, 93.4%)
$\Delta$ S'(cm/s)	$\geq 0.5$	61.7% (46.4%, 75.5%)	52.7% (44.4%, 60.9%)	0.6018 (0.5084, 0.6953)	29.0% (20.4%, 38.9%)	76.1% (72.3%, 88.6%)

**Table 6. 1:** ROC analysis for conventional echocardiographic parameters

$\Delta$ MAPSE: Reduction of mitral annular plane systolic exertion;  $\Delta$ S': Reduction of mitral annular tissue Doppler peak systolic velocity.

\* Empirical cutoff point estimated using nearest to (0,1) method

**Figure 6.5** shows a patient 's echocardiographic parameters of GLS, MAPSE and S' at the baseline and 2<sup>nd</sup> visit. The patient is found to have subclinical ATRCD by  $\Delta$ GLS ( $\geq 15\%$ ),  $\Delta$ MAPSE ( $\geq 2\text{mm}$ ) and  $\Delta$ S' ( $\geq 0.5\text{cm/s}$ ).



**Figure 6. 5:** A patient ‘s echocardiographic parameters of GLS, MAPSE and S’ at the baseline and 2nd visit. The patient is found to have subclinical ATRCD by  $\Delta$ GLS ( $\geq 15\%$ ),  $\Delta$ MAPSE ( $\geq 2$ mm) and  $\Delta$ S’ ( $\geq 0.5$ cm/s).

Left (top to bottom): Baseline’s GLS, MAPSE medial, MAPSE lateral, S’ medial and S’ lateral.

Right (top to bottom): 2<sup>nd</sup> visit’s GLS, MAPSE medial, MAPSE lateral, S’ medial and S’ lateral.

## **6.5 Discussion**

In the present sub study we assessed the role of conventional echocardiographic parameters (MAPSE and S') on detecting Subclinical ATRCD. Our results showed fairly good accuracy of both test.

### **6.5.1 Modalities for assessing LV longitudinal function and their advantages/disadvantages**

Subclinical ATRCD has been defined as a relatively reduction of GLS  $\geq 15\%$  compare to baseline (Plana et al., 2014). GLS measured by STE has been proved to be more sensitive than LVEF, as it decreases preceding the significant reduction of LVEF in patients treated with anthracycline therapy (Sawaya et al., 2012, Thavendiranathan et al., 2014). GLS represents the LV longitudinal systolic function that is the most important component of LV global systolic function. Other components of LV systolic function include radial contraction and circumferential twisting (Smiseth et al., 2016).

Left ventricular longitudinal function can be impaired in the presence of a normal LVEF, and this has been demonstrated using a number of echocardiographic parameters including GLS by STE (Carasso et al., 2008, Liu et al., 2009, Kraigher-Krainer et al., 2014, Shah et al., 2015), MAPSE by M-mode (Höglund et al., 1988, Wenzelburger et al., 2011) and S' by TDI (Yip et al., 2002, Yu et al., 2002, Petrie et al., 2002, Vinereanu et al., 2003). MAPSE, representing the longitudinal motion of the mitral annular plane, is the distance of horizontal displacement of mitral annulus between the end diastole and peak systole. S' obtained by TDI is another parameter that represents the longitudinal motion of the mitral annular plane. Deferent from MAPSE, S' is the peak systolic velocity of the mitral annulus.

Suggested advantages of GLS over MAPSE and S' variables include its relative independence from translation, tethering and the angle of incidence. However, there are also limitations of

GLS, with a major one being that accurate measurement is dependent on having non-foreshortened LV imaging in all three apical LV views, in conjunction with imaging of adequate quality to allow tracking in most of the 6 segments in each of these views. Indeed, in large studies in subjects with normal LVEF, GLS has not been feasible in a substantial proportion of the subjects (Kraigher-Krainer et al., 2014, Shah et al., 2015, Biering-Sørensen et al., 2017), with the implication that it cannot be used for the assessment of LV longitudinal function in all individuals. Inadequate imaging quality for strain measurement was reported in 56/477 subjects by Shah et al (Shah et al., 2015), and in 82/301 subjects by Kraigher-Krainer et al (Kraigher-Krainer et al., 2014). Furthermore, in one population study, 858/2154 subjects were excluded from strain measurement because of inadequate frame rate or inadequate image quality, and in the remaining 1296 subjects strain could be measured in all 3 apical projections in only 284 subjects (Biering-Sørensen et al., 2017). In our study group, GLS measurement was not feasible in 25/383 (6.5%) patients at the initial screening stage. In contrast, MAPSE and S' could be made in all subjects in the present study. This consistent with the reported high feasibility of MAPSE and S' measurements in population studies (Dalen et al., 2010, Støylen et al., 2020, Støylen et al., 2018, Mogelvang et al., 2015). On the other hand, the limited availability of STE has been the biggest hinder of its usefulness in Uganda. The present study compared the two more feasible echocardiographic parameters (MAPSE/S') with GLS to assess their role on detecting subclinical ATRCD.

### **6.5.2 Correlation between GLS and MAPSE/S'**

We first assessed the correlation between GLS and MAPSE/S'. The presence of positive correlations of GLS with MAPSE and S' in our study are consistent with the findings of previous studies (Peverill and Cheng, 2020, Wenzelburger et al., 2011, Kraigher-Krainer et al., 2014,

Shah et al., 2015, Carluccio et al., 2011). The observation of a closer relationship between GLS and MAPSE ( $r=0.376$ ) compared to that between GLS and  $S'$  ( $r=0.233$ ) was also reported by Roger E et al. (Peverill and Cheng, 2020). This may be explained by MAPSE being a distance parameter, can be a better surrogate of GLS – a parameter of relative change of myocardial length, than  $S'$  which is a velocity parameter.

### **6.5.3 Use $S'$ to detect subclinical LV systolic dysfunction**

To define the ability of MAPSE/ $S'$  to detect subclinical ATRCD, ROC analysis was done to compare these conventional echocardiographic parameters with GLS. Considering MAPSE,  $S'$  and GLS have similar biological variability (declined with age) (Støylen et al., 2020, Støylen et al., 2018) and  $\Delta$ GLS is used in the guideline (Plana et al., 2014) (Lyon et al., 2022), we studied  $\Delta$ MAPSE/ $\Delta S'$  as the variables of the test to be investigated. Our results showed fairly good accuracy on both  $\Delta$ MAPSE and  $\Delta S'$ .

Several studies demonstrated the usefulness of  $S'$  in detecting subclinical LV systolic dysfunction in different clinical settings. Study published by Vinereanu D et al. in 2003 and Sameeh et al. in 2018 suggested measurement of  $S'$  may be a more sensitive marker of subclinical changes in LV performance in diabetes than assessment of global function by LVEF (Vinereanu et al., 2003, Raafat et al., 2018). However, both studies didn't compare  $S'$  with GLS. In the setting of cancer population, Nazanin Fallah-Rad et al. enrolled 42 breast cancer patients who underwent anthracycline plus trastuzumab therapy. They found a significantly reduced  $S'$  (cutoff of 0.6cm/s) detected as early as 3 months after chemotherapy seems to predict a decline in LVEF after 6 months with high sensitivity (93%) and specificity (99%) (Fallah-Rad et al., 2011). This result is very close to our study finding regarding the  $S'$  cutoff of 0.5cm/s. The different study methods could have contributed to the different sensitivity and specificity of  $S'$ ,

since we used GLS as the reference test for assessment but not the case in Nazanin Fallah study, which compared S' with LVEF. Zhang et al. investigated 82 patients with diffuse large B-cell lymphoma treated with anthracycline based chemotherapy and reported S' < 13.65 cm/s (sensitivity, 66.7%; specificity, 71%; AUC = 0.682) after 2-4 chemotherapy cycles from the baseline values can reliably predict cardiotoxicity (Zhang et al., 2017). Although the accuracy of S' in this study is similar with our study, however, other than our study that  $\Delta S'$  was tested, they tested the value of S'. There is also report of greater sensitivity of S' compared with LVEF for detection of early anthracycline toxicity by Florescu group (Florescu et al., 2014), but no cutoff value of S' was defined in this study.

#### **6.5.4 Use MAPSE to detect subclinical LV systolic dysfunction**

Previous studies have also demonstrated the role of MAPSE in various cardiovascular diseases. Reduced MAPSE can be used as a sensitive early marker of LV systolic dysfunction in hypertension (Xiao et al., 2006), diabetes (Magdy G, 2017), coronary artery disease (Willenheimer et al., 2002) and aortic stenosis (Herrmann et al., 2011, Rydberg et al., 2004, Takeda et al., 2001). MAPSE has been described as a useful and easily acquired measurement, especially on exercise, for the diagnosis of heart failure patients with preserved EF (Wenzelburger et al., 2011). Present study assessed the role of MAPSE in detecting subclinical ATRCD, an area has received little attention in previous studies.

We found  $\Delta\text{MAPSE} \geq 2\text{mm}$  was able to detect subclinical ATRCD with AUC of 0.6736, sensitivity of 74.47% and specificity of 54.9%.

It is to be noted that our results showed both  $\Delta\text{MAPSE} \geq 2\text{mm}$  and  $\Delta S' \geq 0.5\text{cm/s}$  had a higher NPV (87.5% and 76.1%) than PPV (33.7% and 29.0%), which underlining these parameters can serve as a screening test for subclinical ATRCD when GLS by STE is not available or feasible.

The sensitivity and NPV are further improved when the two test are used as simultaneous (parallel) test.

## **6.6 Conclusion**

MAPSE and S' have a significant correlation with absolute GLS. The  $\Delta$ MAPSE and  $\Delta$ S' showed fairly good accuracy, sensitivity and negative predictive value to detect subclinical ATRCD in Ugandan cancer patients. These simple conventional echocardiographic parameters may serve as screening tools for detecting subclinical ATRCD in resource limited settings where GLS by STE is not available or not feasible. Both test maybe measured simultaneously whenever possible in order to improve the sensitivity and NPV.

## **Chapter 7: Lipoprotein subfractions pattern before and after anthracycline therapy in breast cancer patients with low baseline cardiovascular risk – A pilot study**

### **7.1 Introduction**

Anthracycline therapy's impact on cardiovascular health has traditionally been its directly causing cardiac toxicity and various degree of cardiac dysfunction, namely ATRCD. Recent in vitro and in vivo studies done on anthracycline treated animal models and humans showed anthracycline therapy leads to dyslipidemia, thus alters cardiovascular risks in cancer survivors (Ma et al., 2002, He et al., 2020, Mangiapane, 1990). Anthracycline therapy related dyslipidemia is associated with increased non HDL (VLDL, IDL, LDL) and decreased HDL (Dong et al., 2022, Tian et al., 2019, Xu et al., 2020). These research findings have drawn growing attentions on anthracycline 's action on lipid hemostasis. As many anthracycline treated cancer patients survive longer, it is reasonable to concern the potential added cardiovascular risk brought by this therapy.

It is evident high non HDL and low HDL are the major risk factors for cardiovascular disease (Bachorik and Ross, 1995, Warnick and Wood, 1995). Traditionally, blood lipoprotein level measurements have been used clinically for risk classification and treatment guidance. However, it has limited benefits in patients who developed cardiovascular events with “normal lipoprotein level” (Sachdeva et al., 2009). LDL can be further divided into seven subclasses depending on its particle size using gel electrophoresis methods by Quantimetrix Lipoprint® System, with LDL 1 is the largest and LDL 7 is the smallest subfraction. The smaller the LDL size the more atherogenic it is (Rizzo and Berneis, 2006). Base on that, LDL subfractions is defined by two patterns: Pattern A consists predominately larger, buoyant LDL particles and Pattern B consist

predominately smaller and denser LDL particles (Austin et al., 1988, St-Pierre et al., 2005). The clinical implication for the classification is that Pattern B is associated with increased cardiovascular risk (St-Pierre et al., 2005, Austin et al., 1988).

How anthracycline therapy affects LDL subfractions has never been studied. We hypothesize that anthracycline alters lipid homeostasis manifested by changing the lipoprotein subfractions, particularly LDL subfractions. We aim to investigate the changes of lipoprotein subfractions after anthracycline therapy on selected breast cancer patients with no pre-existing cardiovascular risk at the baseline.

## **7.2 Method**

This is a sub study nested from the main cohort- SATRACD study (Detecting subclinical anthracycline therapy related cardiac dysfunction in low income country). The detailed methodology of the main study is stated in chapter 3. In this pilot study, no pre-defined sample size was used. All the main study patients who met the inclusion criteria of the pilot study were included.

### **7.2.1 Inclusion and exclusion criteria**

The inclusion criteria for the sub study are 18 to 49 year-old, being diagnosed with breast cancer, completed anthracycline based chemotherapy and paid for the 2<sup>nd</sup> visit at the study site with fasting blood samples at the baseline and 2<sup>nd</sup> visit being obtained. Patients who had recognized pre-existing cardiovascular risk factors (including: hypertension, diabetes mellitus, chronic kidney disease, obesity, smoking and HIV infection) at the enrollment or had received chest radiotherapy before the 2<sup>nd</sup> visit were excluded.

### 7.2.2 Data acquisition

The demographic and clinical data for eligible patients were extracted from the main pool of the cohort. Their serum samples collected at baseline and 2<sup>nd</sup> visits (ending of anthracycline therapy) were identified from the main study blood samples for lipoprotein subfractions analysis at the laboratory of Cape heart institute, University of Cape Town, where they were stored at -80 centigrade.

#### LDL subfractions measurement

LDL subfractions analysis was performed using the Quantimetrix Lipoprint<sup>®</sup> System and LDL Subfractions Kit (**Figure 7.1**) with the principle of gel electrophoresis. The Lipoprint LDL Kit consists of precast, high resolution polyacrylamide gel tubes, a loading gel solution containing a lipophilic dye and the buffer salts.



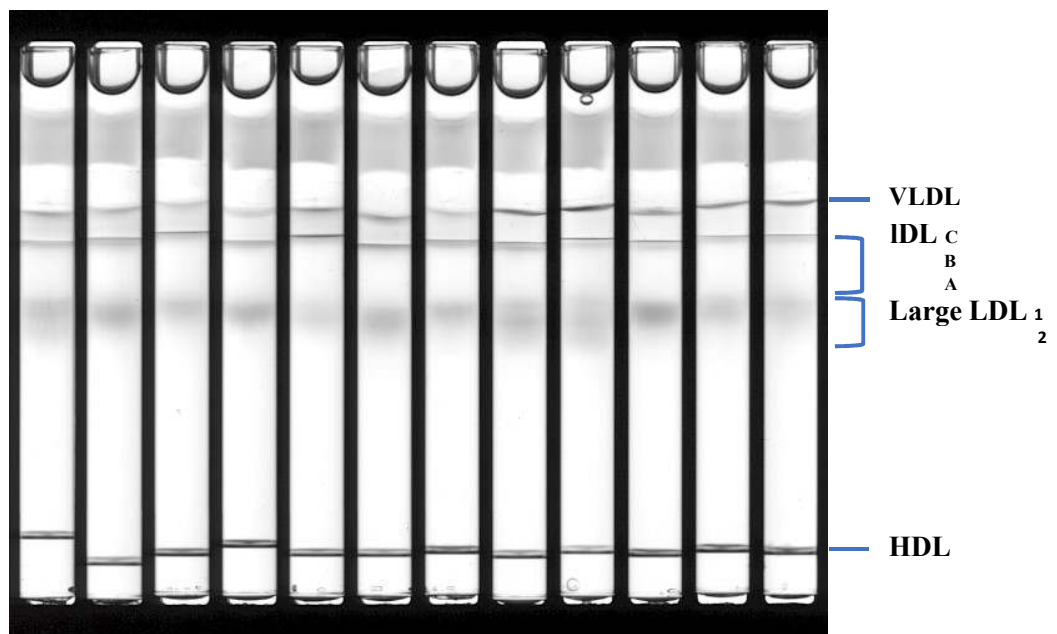
**Figure 7. 1:**Quantimetrix Lipoprint<sup>®</sup> System and LDL Subfractions Kit

1: Gel tube; 2: loading gel solution; 3: Preparation rack; 4: Preparation light; 5: Electrophoresis chamber; 6: Power source; 7: Scanner; 8: Computer system with Lipoware Analysis Program

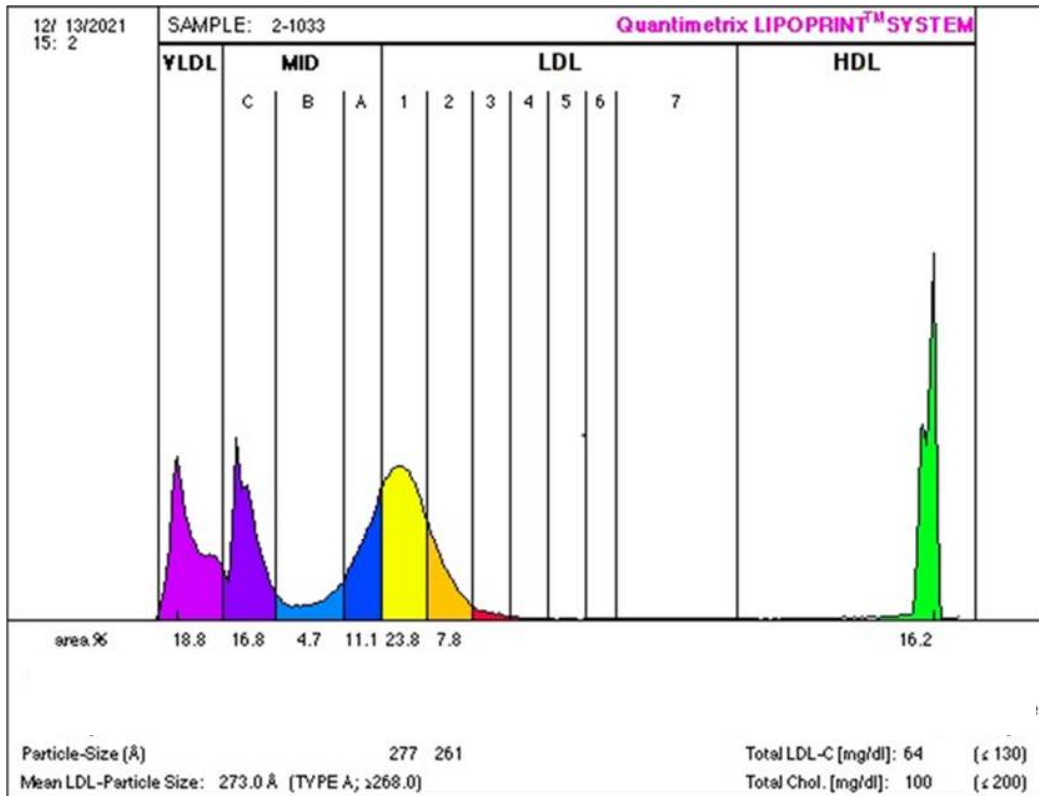
The assay procedure includes: 1. Prepare the electrolyte buffer solution by dissolving one vial of the buffer salts in 1200 mL of deionized/distilled water. 2.Place the gel tubs in the Preparation

Rack with the unfilled end up. 3. Remove the storage buffer completely from the top of the gels by shaking the rack while inverted. 4. Apply 25 $\mu$ L of serum to each tube. 5. Add 200 $\mu$ L of Lipoprint Loading gel to each tube. 5. Mix the loading Gel with the specimen by inverting the Preparation rack several times. 6. Photo polymerize the Loading Gels for 30 minutes with the Preparation Light. 7. Insert each gel tube into the upper chamber of the electrophoresis chamber. 8. Place 1000 mL of electrolyte buffer solution in the lower chamber and 200 mL in the upper chamber. 9. Electrophoresis with the current of 3 mA per each Gel Tube for about 60 minutes until the HDL fraction is about 1 cm from the bottom of the fastest migrating Gel Tube. 10. Transport the gel tubes from the electrophoresis chamber to the scanner for analysis.

Quantitative results were analyzed in computer by Lipoware Analysis Program. The Gel Tubes were scanned (**Figure 7.2**) and the relative area of each lipoprotein subfractions was established by dropping vertical lines at predetermined cut-off ranges for each band. (**Figure 7.3**)



**Figure 7. 2:** Scan of the gel tube shows the lipoprotein subfractions distributions for 12 individuals



**Figure 7.3:** Quantification of the lipoprotein subfractions by the relative area under the curve

The following lipoprotein subfractions were measured in a descending order of the particle size:

VLDL, IDL-C, IDL-B, IDL-A, LDL-1 to 7 and HDL. The mean LDL particle diameter and subfractions pattern were also defined by the computer system. Pattern A contains predominately large LDL (LDL-1 and LDL-2) with a mean particle diameter  $\geq 26.5$  nm. Pattern B contains predominantly small LDL (LDL-3 to LDL-7) with a mean particle diameter  $< 26.5$  nm.

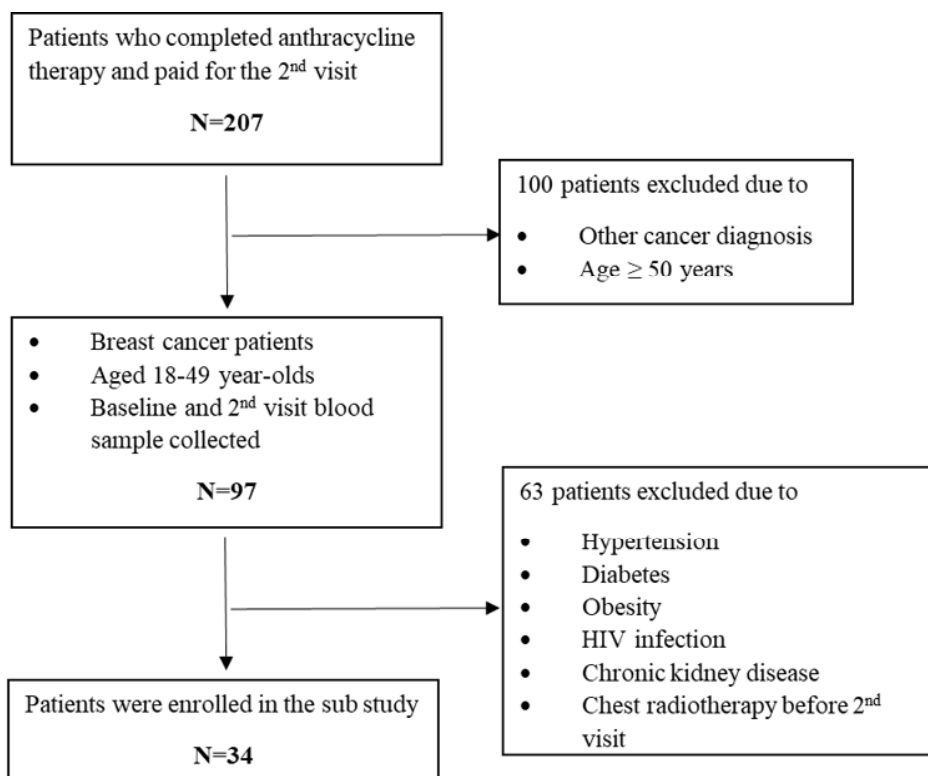
### 7.3 Statistic analysis

We analyzed the data using STATA v14 (Institute Inc., College Station, TX, USA). All continuous variables were expressed as a mean  $\pm$  SD and categorical variables as frequencies and percentages. Paired t test (for Gaussian distribution) and Wilcoxon signed rank test (for non-Gaussian distribution) were used to compare dependent continuous variables, where appropriate.

Pearson test was used to assess the correlation between the continuous variables. Simple linear regression mode was made to test the relationship of the continuous outcome and one continuous variable. Two-sided p-value <0.05 was considered statistically significant.

## 7.4 Results

In total, 34 eligible patients were enrolled in the sub study (**Figure 7.4**). They were all females with a mean age of  $38 \pm 7$ , 16 (47.1%) had stage 3 and 7 (20.6%) had stage 4 diseases. All the patients received 6 circles of anthracycline (doxorubicin), cyclophosphamide and fluorouracil combination therapy with the average cumulative anthracycline dose of  $320.7 \pm 37.2 \text{ mg/m}^2$ . There were 9 (26.5%) patients were diagnosed with subclinical ATRCD at the end of anthracycline therapy, the rest had no evidence of anthracycline cardiotoxicity and none of the 34 patients had clinical ATRCD on 2<sup>nd</sup> visit.



**Figure 7. 4:** Patients’ flow chart for lipid sub study

### 7.4.1 Lipoprotein subfractions at the baseline and ending of anthracycline therapy

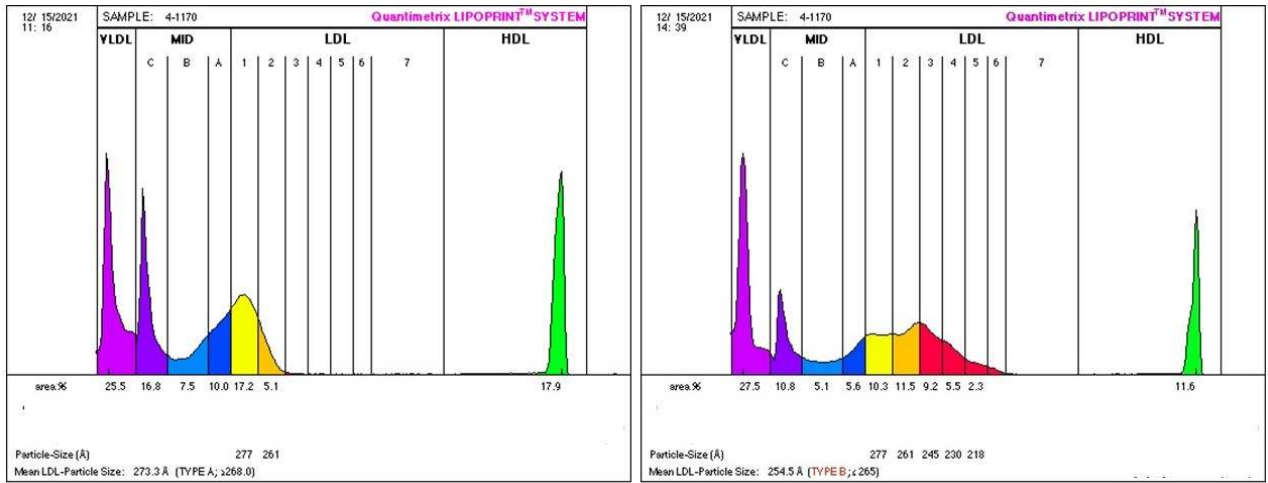
Patients' examination parameters at the baseline and 2<sup>nd</sup> visit were compared and no significant changes were found regarding body weight, BMI, systolic blood pressure and diastolic blood pressure. (Table 7.1)

Lipoprotein subfractions results at baseline and 2<sup>nd</sup> visit are presented and compared in the same table. We found a significant increase in VLDL subfraction after anthracycline therapy. This only holds true among patients without evidence of anthracycline cardiotoxicity. There were no significant changes in all the IDL, LDL subfractions and HDL subfraction. Mean LDL particle size was significantly reduced after anthracycline therapy, particularly in patients who had no evidence of anthracycline cardiotoxicity. At the baseline, all the patients had LDL subfractions Pattern A. At the completion of anthracycline therapy, 5 (14.7%) patients shifted to Pattern B, with 4 out 5 Pattern B patients belonged to normal group.

	All patients (N=34)			Normal patients (n=25)			Subclinical ATRCD patients (n=9)		
	Baseline	Completion	p value	Baseline	Completion	P value	Baseline	Completion	P value
<b>Physical examination</b>									
Wight (kg, mean±SD)	61.5±10.5	61.4±10.6	0.8686	60.1±10.7	59.9±10.5	0.7904	65.6±9.4	65.7±10.3	0.9195
BMI (Kg/m <sup>2</sup> , mean±SD)	23.8±3.5	23.8±3.4	0.921	23.2±3.5	23.1±3.3	0.8201	25.4±3.1	25.5±2.9	0.8531
SBP (mmHg, mean±SD)	122.4±13.7	123.6±13.1	0.5773	122.6±14.6	122.7±13.7	0.961	121.8±11.7	125.8±11.9	0.3194
DBP (mmHg, mean±SD)	76.0±11.9	76.5±9.7	0.8289	74.4±13.1	74.7±9.7	0.9253	80.3±7.2	81.4±8.3	0.8067
<b>Lipoprotein subfractions</b>									
VLDL (% ,mean±SD)	19.9±3.13	21.5±3.28	<b>0.0148*</b>	19.5 3.2	20.9 2.9	<b>0.0293*</b>	21.1 2.7	23.0 4.0	0.2604*
IDL-C (% ,mean±SD)	13.5±2.5	12.8±2.5	0.1704	13.5 2.7	12.4 2.6	0.071	13.3 2.1	13.7 1.9	0.6912
IDL-B (% ,mean±SD)	5.5±1.6	6.2±3.1	0.2123	5.4 1.6	6.4 3.3	0.2027	5.8 1.7	5.9 2.6	0.8647
IDL-A (% ,mean±SD)	9.9±2.8	9.5±3.1	0.5018	9.6 2.7	9.9 2.9	0.7343	10.6 3.3	8.3 3.3	0.1259
LDL-1 (% ,mean±SD)	20.0±3.8	18.3±4.2	0.0549	20.4 4.2	18.6 3.8	0.0929	18.8 2.6	17.4 5.4	0.3977
LDL-2 (% ,mean±SD)	8.0±3.8	8.5±4.5	0.5422	8.5 3.9	8.8 4.3	0.7405	6.7 3.2	7.7 5.3	0.5832
LDL-3 (% ,mean±SD)	1.4±2.9	1.8±2.7	0.4471*	1.7 3.3	1.8 2.6	0.684*	0.6 1.0	1.9 1.0	0.4297*
LDL-4 (% ,mean±SD)	0.1±0.8	0.3±1.1	0.1894*	0.2 0.9	0.2 0.7	0.3497*	0	0.6 1.8	0.3173*
LDL-5 (% ,mean±SD)	0	0.1±0.4							
LDL-6 (% ,mean±SD)	0	0							
LDL-7 (% ,mean±SD)	0	0							
HDL (% ,mean±SD)	21.5±4.2	20.6±4.7	0.1858	20.9±4.3	20.5±4.9	0.5696	22.9±3.8	20.8±4.3	0.0793
<b>Average LDL particle size (nm, mean±SD)</b>	27.2±0.2	26.9±0.5	<b>0.0369</b>	27.2±0.2	26.9±0.5	<b>0.0468</b>	27.3±0.2	27.0±0.7	0.4119
<b>LDL Subfraction Tpye B (n,%)</b>	0, 0	5, 14.7%		0,0	4,16%		0,0	1,11%	

**Table 7. 1:** Patients physical examination and lipoprotein subfractions

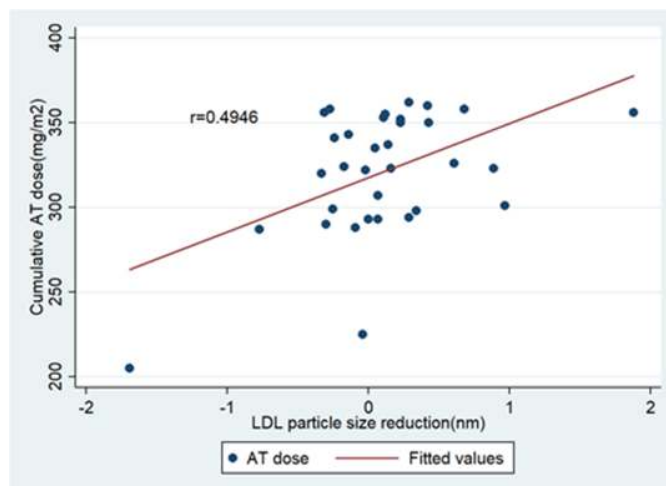
\*: p value calculated using Wilcoxon Signed-Rank test



**Figure 7. 5** One patients’ the lipoprotein subfractions analysis graphs  
 Left: Baseline graph shows LDL subfractions Pattern A (mean LDL size:27.3nm);  
 Right: 2<sup>nd</sup> visit graph shows LDL subfractions Pattern B (mean LDL size: 25.4nm).

#### 7.4.2 Correlation between anthracycline dose and LDL particle size reduction

Pearson correlation test shows moderate positive correlation ( $r=0.4964$ ,  $p=0.0034$ ) between the mean LDL particle size reduction and cumulative dose of anthracycline (**Figure 7.6**). Simple linear regression mode was made and showed cumulative anthracycline dose increment was associated with the mean LDL particle size reduction with coefficient of 0.008 and 95% confident interval of 0.003 to 0.126 ( $p=0.003$ ).



**Figure 7. 6:** Correlation between cumulative anthracycline dose and LDL particle size reduction

## **7.5 Discussion**

We carried out this lipoprotein subfractions analysis on a small cohort of homogenous breast cancer patients who underwent the same anthracycline- based chemotherapy with no identified cardiovascular risk factor at the baseline, aiming to investigate if anthracycline therapy affect patients' lipoprotein subfractions. This is a pilot study with interesting results.

### **7.5.1 Increased VLDL subfraction after anthracycline therapy**

There are many studies has reported increased VLDL, LDL and reduced HDL level following anthracycline therapy and most of them studied breast cancer patients (He et al., 2020, Dong et al., 2022, Tian et al., 2019). However, no study has investigated the change of lipoprotein subfractions in anthracycline exposed population. Our results didn't show any significant changes for IDL, LDL and HDL subfractions. But we did find a significantly increased VLDL subfraction after anthracycline based chemotherapy. More interestingly, this VLDL subfraction increment only presented in patients who had no evidence of anthracycline cardiotoxicity.

VLDL belong to the family of ApoB (primarily ApoB100) lipoprotein, which is commonly called non-HDL clinically. Other members of ApoB lipoproteins include IDL and LDL (Sharadendu Bali, 2019). VLDL is primarily produced and secreted from the liver, which represents the beginning of the endogenous lipid metabolism pathway. VLDL is triglyceride rich and have the largest particle size. It is the precursor of IDL and LDL. Overweight and obesity are associated with increased VLDL production (Mittendorfer et al., 2016). In the present study, no significant body weight or BMI changes were observed. This might mean the increase of VLDL subfraction was independent of weight gain but rather related to the anthracycline therapy. Anthracycline (specially doxorubicin) has been proposed to increases the expression of ApoB100 without decreasing the LDL receptor in liver cells, thus increase the production of

ApoB lipoproteins with a normal clearance of LDL (Sharma et al., 2016). This theory supports our findings of increased VLDL subfraction but not LDL subfractions. Moreover, Bjorn Redfors and colleagues' study on rats suggested myocardial cell overexpression of ApoB100 is cardioprotective and anthracycline (specifically doxorubicin) related cardiac dysfunction is associated with decreased myocardial ApoB100 expression. It might be explained that the patients who developed subclinical ATRCD didn't have increased ApoB expression, thus didn't show VLDL subfraction increase. If this is true, then VLDL or VLDL subfraction could be a marker of cardioprotection in anthracycline treated patients. More research is needed to explore on ApoB/VLDL and ATRCD.

#### **7.5.2 VLDL and cardiovascular risk**

High non-HDL has been proved to be associated with high incidence of cardiovascular disease (Bachorik and Ross, 1995). High VLDL particularly has been found to be independently associated with the presence of carotid plaques (Iannuzzi et al., 2018, Abi-Ayad et al., 2018) after adjusting the main cardiovascular risk. Marco Gentile and colleague recently reported carotid intima-media thickness, a marker of cardiovascular disease, is positively correlate with VLDL level, but not other lipoprotein level (Gentile et al., 2020). The results of Jupiter trial showed the smaller VLDL subfraction were associated with cardiovascular risk in the context of normal LDL (Lawler et al., 2017). It is need to be noted that, no matter our findings of increased VLDL subfraction might indicate anthracycline therapy increases patients cardiovascular risk, we didn't measure the absolute lipoprotein levels and subfractions of VLDL subclass as the other investigators did. Therefore, further better designed studies are needed to verify the findings of our study.

### **7.5.3 LDL subfractions patterns after anthracycline therapy**

We found the patients' mean LDL particle size significantly reduced at the end of anthracycline therapy and LDL subfractions Pattern B prevalence raised from 0 to 14.7%. Not only that, the cumulative anthracycline dose is moderately correlated with the mean LDL particle size reduction and cumulative anthracycline dose increment was associated with the mean LDL size reduction. From these findings we could probably propose a causative link between anthracycline and LDL subfraction Pattern B. Rear literature is found to support our observations directly, as no previous research has studied how anthracycline could affect the LDL particle size or LDL subfractions pattern. Nevertheless, studies carried out in various disease settings to investigate the association of oxidative stress biomarkers and LDL phenotype showed the evidences that oxidative stress is an important pathway to cause the smaller LDL particle size and pattern B LDL subfractions (Vekic et al., 2007, Ivanišević et al., 2018, King et al., 2011, Parikh et al., 2015, Kotani et al., 2012). In the cell, anthracycline targets topoisomerase II, which is responsible in DNA replication, triggering profound changes in the transcription leading to defective mitochondrial biogenesis and reduced levels of anti-oxidative enzymes, manifested as increased production of reactive oxygen species and cell death (Zhang et al., 2012).

Anthracycline therapy induced oxidative stress has been believed the main mechanism of ATRCD. Whether this mechanism also explains the generation of small LDL particles need to be further studied.

### **7.5.4 LDL subfractions patterns and cardiovascular risk**

LDL particle size and its subfractions pattern have attracted many researchers' attention, due to its cardiovascular health implications. LDL particle size is suggested an emerging predictor of cardiovascular events and coronary artery disease progression, and National Cholesterol

Education Program Adult Treatment Panel III has proposed dense LDL a novel cardiovascular risk factor (2002). Theoretically, smaller, denser LDL is more atherogenic attribute to its characteristics, such as longer clearing time in plasma, oxidative stress enhancement, more easily binding to and penetrating arterial tissue than larger LDL particle (Krauss, 1994).

LDL subfractions Pattern B, which is smaller, denser LDL particle predominant, is reported independently associated with higher rate of cardiovascular disease progression by several studies (Austin et al., 1988, St-Pierre et al., 2005). However, the role of LDL subfractions in cardiovascular risk stratification is still debatable, since the results from other different studies are largely controversial (Sacks and Campos, 2003, Rizzo and Berneis, 2006). Whether our observation of LDL subfractions pattern shift after anthracycline therapy indicates the alteration of cardiovascular risk cannot be fully answered by this study.

## **7.6 Limitations**

This is a pilot study, which has insufficient power due to a small sample size. Certain degree of selection bias may exist, as only patients with breast cancer of younger age and low cardiovascular risk were included in the study. We also note that serum lipoprotein concentration, which is the standard test for risk assessment was not measured in this study because of limited study resources. This can limit the interpretation of the study results.

Moreover, other potential bias can result from unmeasured or unadjusted confounders, such as, some factors which can affect lipid profiles (diet and weight change during chemo therapy, other chemotherapy regimens or medicine co-administrated with anthracycline).

## **7.7 Conclusion and future perspective**

This pilot study demonstrates significant VLDL subfractions increase, mean LDL particle size reduction and LDL subfractions pattern shift following the completion of anthracycline based

chemotherapy. Cumulative dose of anthracycline is associated with the degree of LDL particle size reduction. Anthracycline therapy might alter patients cardiovascular risk. Despite several limitations, this is the first study that investigated the changes of lipoprotein subfractions on anthracycline treated patients. The findings are not only interesting but also enlightening with areas needed to be further explored by better designed and powered researches that may lead to better understanding of the impact of anthracycline therapy on cardiovascular health of the cancer survivors.

## **Chapter 8: Conclusion and the way forward**

Anthracycline plays a major role in chemotherapy among Ugandan cancer patients, because it is highly effective for treating many type of cancers and relatively affordable. ATRCD is the most common cardiac side effect results from anthracycline therapy. In Uganda, patients with ATRCD is not uncommon and present with advanced heart failure. The poor outcome of ATRCD patients in Uganda is largely due to lack of a good mechanism of cardiac monitoring to detect the disease at subclinical stage and prevent the progression of the disease by prioritizing the limited resource to high risk patients. “A journey of thousand miles begins with a single step.” We believe the first step to fill the gap is the locally generated research data, showing us where we are.

### **8.1 Key findings and their clinical/public health implications**

#### **Incidence, predictors of ATRCD and using MAPSE/S' to detect subclinical ATRCD**

The findings of this study showed a high incidence of ATRCD in Ugandan cancer patients. Most of them are at the subclinical stage or asymptomatic clinical stage. These findings clearly highlight the importance of cardiac monitoring program, which is lacking in Uganda, for such population who are at risk of stage B heart failure. Without regular cardiac monitoring, these patients will live with these silent risks until they may present with overt heart failure later in their life.

We found the patients who were diagnosed with subclinical ATRCD at the end of the anthracycline therapy and patients with HIV infection were at high risk of developing clinical ATRCD. These findings inform the health workers and cancer patients in Uganda that echocardiographic screening for subclinical ATRCD at the end of the anthracycline therapy should not be omitted, especially in cancer patients who live with HIV.

Moreover, the results of fairly good accuracy and high NPV of  $\Delta$ MAPSE and  $\Delta$ S', suggest these simple echocardiographic parameters can be surrogates of GLS for detecting subclinical ATRCD. These findings provide an easy tool of screening subclinical ATRCD for the clinicians in Uganda, where STE is not readily available.

### **CVD risks before and after anthracycline therapy**

This project was also able to look into the pre-existing CVD risks with the baseline data and potentially added CVD risks after anthracycline therapy with the pilot lipid study.

The baseline data showed hypertension was the most common pre-existing CVD risk factors, followed by obesity and HIV. However, limited by the study power, hypertension and obesity, didn't show significant link with the development of subclinical and clinical ATRCD.

Nevertheless, as the traditional CVD risk factors, the high prevalence of hypertension and obesity in this cancer population should not be overlooked, for the active intervention will lead to a better CVD prevention and outcome in cancer survivors. This, again, emphasizes the need of joint care of oncologist and cardiologist in Uganda.

In the pilot study, we observed that anthracycline therapy reduced mean LDL particle size and 5/34 (14%) of the patients shifted from LDL subfractions Pattern A to the atherogenic Pattern B. Although being limited by the small sample and study design, we still, based on the findings, hypothesize that anthracycline therapy alters lipid homeostasis and increase the CVD risk in cancer survivors. We wish the research on anthracycline 's lipid homeostasis continuous to bring more insight on the cardiovascular health impact of anthracycline therapy.

## **8.2 Future perspectives**

### **Establish evidence based local cardiac monitoring protocol**

Based on these results, we can propose a local cardiac monitoring protocol tailored to the limited resources. We recommend all the patients who receive anthracycline therapy should at least go through echocardiographic screening at the baseline and at the end of the therapy with GLS or MAPSE/S' measured. This is strongly recommended for HIV positive patients.

Echocardiography assessment should be repeated after 6 month of ending anthracycline therapy on selected patients who are defined with subclinical ATRCD with  $\Delta\text{GLS} \geq 15\%$  or  $\Delta\text{MAPSE} \geq 2\text{mm}$  or  $\Delta\text{S}' \geq 0.5\text{cm/s}$ . We strongly recommend HIV positive patients being monitored up to 6 months of ending anthracycline therapy regardless the echocardiographic results at the ending of the therapy.

### **Future researches**

We also realized the limitations of our study and advocate more robust researches on anthracycline's cardiovascular side effects to be carried out in Uganda and SSA population in the near future, aiming to investigate further the risk factors of ATRCD, identify the cost effective cardiac surveillance tools (including ECHO and biomarkers, e.g. TnI and BNP) and protocols, as well as study the cardiovascular outcomes (including lipid profile and subfractions) of patients with or without ATRCD following anthracycline exposure. These objectives are to be achieved with a prospective multi-center cohort study.

Finally, we hope the work of this project will draw more attention from the health workers to improve the cardiovascular care of cancer patients in Africa and help to initiating the cardio-oncology services in Uganda and Africa.

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## Appendices

### ESC Cardio-oncology 2022 guideline (Lyon et al.,2022) ATRCD definition and the terms used in this thesis

ESC cardio-oncology Guideline 2022 Definition			Terms used in this thesis
Asymptomatic ATRCD	Mild	LVEF $\geq$ 50% AND new relative decline in GLS by >15% from baseline AND/OR new rise in cardiac biomarkers	Subclinical ATRCD
	Moderate	New LVEF reduction by $\geq$ 10 percentage points to an LVEF of 40–49% OR New LVEF reduction by <10 percentage points to an LVEF of 40-49% AND either new relative decline in GLS by >15% from baseline OR new rise in cardiac biomarkers	
	Severe	New LVEF reduction to ,40%	
Symptomatic ATRCD	Mild	Mild HF symptoms, no intensification of therapy required	Clinical ATRCD
	Moderate	Need for outpatient intensification of diuretic and HF therapy	
	Severe	HF hospitalization	
	Very severe	HF requiring inotropic support, mechanical circulatory support, or consideration of transplantation	

**Cardiovascular Topics**

**Subclinical anthracycline therapy-related cardiac dysfunction: an ignored stage B heart failure in an African population**

Wan Zhu Zhang, Feriel Azibani, Karen Sliwa

**Abstract**

Anthracyclines are potent antineoplastic agents with a proven efficacy in the treatment of many paediatric and adult haematological and solid-organ cancers. Anthracycline therapy-related cardiac dysfunction (ATRCDD) is the commonest and most well-studied chemotherapy-induced cardiovascular toxicity. Therefore patients who received anthracycline therapy are considered in stage A heart failure. Recent study findings suggest that anthracycline cardiotoxicity represents a continuum that begins with subclinical myocardial cell injury, followed by an early asymptomatic decline in left ventricular ejection fraction that can progress to symptomatic heart failure if left untreated. In Western countries, ATRCD has been reported in 57% of anthracyclines-treated patients. However, data on incidence and spectrum of ATRCD in Africa are not available. This literature review aimed to highlight the concept of subclinical ATRCD as a stage B heart failure in the spectrum of ATRCD, and the importance of early detection. We emphasise the potential burden and risk of subclinical ATRCD in the African population, with the ultimate aim of drawing the attention of health workers in Africa to improve care of the relevant population.

**Keywords:** subclinical anthracycline therapy-related cardiac dysfunction, stage B heart failure, African population

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Anthracyclines are potent antineoplastic agents with proven efficacy in the treatment of many paediatric and adult haematological and solid-organ cancers. Anthracycline therapy-related cardiac dysfunction (ATRCDD) is the most notorious and well-studied chemotherapy-induced cardiovascular toxicity.

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This dose-dependent ATRCD was first described in 1971 in a cohort of 67 patients treated with Adriamycin for a variety of tumours.<sup>1</sup> The clinical significance of anthracycline cardiotoxicity is growing with the increasing number of cancer survivors worldwide. ATRCD is defined as a decrease in left ventricular ejection fraction (LVEF) of > 10%, to a value < 53%.<sup>2</sup>

Anthracycline toxicity may be acute, early or late. Acute toxicity, which develops in 1% of patients immediately after infusion, is uncommon and generally reversible.<sup>3</sup> Early effects occur within the first year of treatment, while late effects manifest after several years (median of seven years after treatment).<sup>4,5</sup> Early- and late-onset cardiac dysfunction are more likely to be irreversible.<sup>6</sup>

In the literature there is wide variation in the reported frequency of clinical cardiotoxicity. Differences in study population, treatment protocols and duration of follow up could account for this wide variability. The prevalence of late asymptomatic ATRCD has been reported to be more than 57% at a median of 6.4 years after treatment among survivors of childhood cancers,<sup>6</sup> and the incidence of symptomatic heart failure as high as 16%, 0.9 to 4.8 years after treatment.<sup>7</sup>

According to the American College of Cardiology and American Heart Association guidelines,<sup>8</sup> patients who received cardiotoxic agents are considered in stage A heart failure. This identifies patients who are at a high risk for developing heart failure with no evidence of cardiac structural disorder. Stage B refers to patients with cardiac structural disorder but who have never developed symptoms of heart failure. Stage C denotes patients with symptoms of heart failure associated with underlying structural heart disease, and stage D designates the patient with end-stage disease who requires specialised treatment strategies such as mechanical circulatory support, continuous inotropic infusions, cardiac transplantation or hospice care. This approach to the classification of heart failure emphasises both the evolution and progression of the disease (Table 1).

**Table 1. Classification of heart failure (HF)**

Stage of HF	Definition
A	High risk for developing HF with no evidence of cardiac structural disorder
B	Cardiac structural disorder but has never developed symptoms of HF
C	Symptoms of HF associated with underlying structural heart disease
D	End-stage disease requiring specialised treatment strategies

## Rational and design of SATRACD study: detecting subclinical anthracycline therapy related cardiac dysfunction in low income country

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### Abstract:

**Background:** Anthracycline therapy-related cardiac dysfunction (ATRCD) is the most notorious adverse side-effect of chemotherapy. It has become a significant cardiovascular health concern for long-term cancer survivors. With the emerging concept of subclinical ATRCD and newer diagnostic tools (Speckle Tracking Echocardiography (STE) and biomarkers), detecting anthracycline cardiac toxicity at an early stage has become an important step to prevent severe cardiac dysfunction and improve the cardiovascular outcome in cancer survivors. Despite the increasing population at risk in sub-Saharan Africa (SSA), there is no contemporary data in Uganda to address the burden, pathogenesis and risk factors of subclinical ATRCD. This big gap in knowledge has led to a lack of local guidelines for monitoring and management of ATRCD.

**Methods:** SATRACD (Detecting Subclinical Anthracycline Therapy Related Cardiac Dysfunction In Low Income Country) study is an observational prospective cohort study. Three hundred and fifty-three anthracycline naïve cancer patients will be recruited at baseline. Patients are followed up on completion of anthracycline-based chemotherapy and at 6 months after completion of anthracycline therapy. Data on demographics, cancer profile and clinical presentation will be collected at baseline. Comprehensive cardiac assessment will be performed at each visit, including electrocardiogram, conventional echocardiography, STE, cardiac and oxidative stress markers. We will be able to determine the incidence of subclinical and clinical ATRCD at 6 months after completion of anthracycline therapy, determine whether hypertension is a major risk factor for ATRCD, evaluate the role of conventional echocardiography parameters, and biomarkers for detecting subclinical ATRCD.

**Conclusion:** This SATRACD study will provide contemporary data on Ugandan cancer patients who have subclinical and clinical ATRCD, help in the development of local strategies to prevent and manage ATRCD, and improve cardiovascular outcome for Ugandan cancer survivors.

**Keywords:** SATRACD study, subclinical anthracycline therapy, cardiac dysfunction, low income country.

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### Introduction

Anthracyclines are potent antineoplastic agents with proven efficacy in the treatment of many pediatric-, adult-hematologic and solid organ cancers. Dose-de-

pendent anthracycline therapy-related cardiac dysfunction (ATRCD) is the most common and well-studied chemotherapy-induced cardiovascular toxicity. It was first described in 1971 in 67 patients treated with Adriamycin for a variety of tumors.<sup>1</sup> The clinical significance of anthracycline cardiotoxicity is increasing with larger numbers of cancer survivorship worldwide.<sup>2</sup> The prevalence of late asymptomatic cardiac damage has been reported to be more than 57%, at a median of 6.4 years after treatment, among survivors of childhood cancers,<sup>3</sup> and the incidence of symptomatic heart failure as high as 16%, 0.9 to 4.8 years after treatment.<sup>4</sup> Risk of mor-

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## RESEARCH ARTICLE

# Clinical characterization, cardiovascular risk factor profile and cardiac strain analysis in a Uganda cancer population: The SATRACD study

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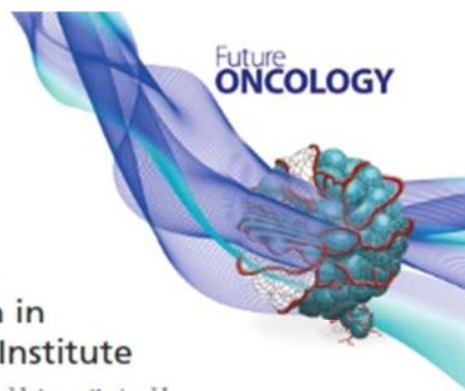
## Abstract

### Background

The link between cancer and cardiovascular disease is firmly established. We sought to investigate the prevalence of cardiovascular disease (CVD) risk factors in Uganda cancer patients, their pre-chemotherapy left ventricular strain echocardiographic pattern and its associations with the CVD risk factors.

### Methods and results

Baseline pre-chemotherapy data of patients who were enrolled in the SATRACD study (a cancer cohort, who were planned for anthracycline therapy), were analyzed. The prevalence of cardiovascular risk factors and baseline strain echocardiographic images were assessed. Among the 355 patients who were recruited over a period of 15 months, 283 (79.7%) were female, with a mean age of 43 years. The types of cancer of the study patients included breast cancer (70.6%), lymphomas, sarcomas, leukemias and hepatocellular carcinoma. Hypertension was the most common comorbidity (27.0%). The prevalence of obesity was 12.1% and that of HIV was 18.3%. All patients had a normal left ventricular ejection fraction (LVEF). The mean global longitudinal strain (GLS) was  $-20.92 \pm 2.43\%$ , with females having a significantly higher GLS than males ( $-21.09 \pm 2.42$  vs  $-20.25 \pm 2.39$ ,  $p = 0.008$ ). Fifty-three patients (14.9%) had suboptimal GLS (absolute  $GLS \leq 18.00\%$ ), which was associated with obesity (POR = 3.07; 95% CI, 1.31–6.98;  $p = 0.003$ ), alcohol use (POR = 1.94; 95% CI, 1.01–3.74;  $p = 0.044$ ), long QTc interval in electrocardiogram (POR = 2.54; 95% CI, 1.06–5.74;  $p = 0.015$ ), and impaired left ventricular relaxation (POR = 2.24; 95% CI, 1.17–4.25;  $p = 0.007$ ). On multivariable logistic regression analysis, obesity (POR = 2.95; 95% CI, 1.24–7.03;  $p = 0.014$ ) was the only independent factor associated with suboptimal GLS.



## Detecting subclinical anthracycline therapy-related cardiac dysfunction in patients attending Uganda Cancer Institute

Wanzhu Zhang<sup>1,2,3\*</sup>, Ferial Azibani<sup>4</sup>, Elena Libhaber<sup>1,5</sup>, Emmy Okello<sup>2,3</sup>, James Kayima<sup>2,3</sup>, Isaac Ssinabulya<sup>2,3</sup>, Joseph Leeta<sup>6</sup>, Jackson Orem<sup>3,6</sup> & Karen Sliwa<sup>1</sup>

<sup>1</sup>Cape Heart Institute, Department of Medicine & Cardiology, Faculty of Health Science, University of Cape Town, Cape Town, 7700, South Africa

<sup>2</sup>Department of Adult Cardiology, Uganda Heart Institute, Kampala, 7051, Uganda

<sup>3</sup>Department of Medicine, College of Health Science, Makerere University, Kampala, 7072, Uganda

<sup>4</sup>UMRS 942 Inserm, Paris, 75010, France

<sup>5</sup>School of Clinical Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, 2050, South Africa

<sup>6</sup>Uganda Cancer Institute, Kampala, 7242, Uganda

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**Aims:** To investigate the incidence of anthracycline therapy-related cardiac dysfunction (ATRC) and its predictors among Ugandan cancer patients. **Patients & methods:** The study recruited 207 cancer patients who were followed for 6 months after ending anthracycline therapy. Global longitudinal strain and troponin-I were the diagnostic tools. **Results & conclusions:** The cumulative incidences of subclinical and clinical ATRCD were 35.0 and 8.8% respectively. The predictors of clinical ATRCD were HIV infection (hazard ratio [HR]: 3.04; 95% CI: 1.26–7.32;  $p = 0.013$ ), lower baseline global longitudinal strain (HR: 0.61; 95% CI: 0.53–0.71;  $p < 0.001$ ) and development of subclinical ATRCD at the end of anthracycline therapy (HR: 6.61; 95% CI: 2.60–16.82;  $p < 0.001$ ). Cardiac surveillance at baseline and at ending of anthracycline therapy is essential to identify high-risk patients.

**Plain language summary:** Anthracyclines are drugs for treating many types of cancers. They may however be harmful to the heart. This anthracycline side effect will first cause subtle heart-cell injury that can be detected and treated if it is handled early. Therefore, this study aims to study patients in the Uganda Cancer Institute to find out how many patients can get and who are likely to get this side effect. We found that 35% of the patients had subtle heart-cell injury and 8.8% had a more severe form of heart-cell injury. The patients who lived with HIV, whose heart was weaker and who got subtle heart-cell injury immediately after treatment were more likely to get the severe form of the side effect. Patients who receive anthracycline therapy need to be monitored closely to prevent serious heart injury.

First draft submitted: 6 April 2022; Accepted for publication: 24 June 2022; Published online: 7 July 2022

**Keywords:** clinical anthracycline-related cardiac dysfunction • incidence • predictor • subclinical anthracycline therapy-related cardiac dysfunction

The economic and lifestyle transitions in sub-Saharan Africa have brought not only population growth and aging but also paradigm shifts of disease epidemiology from infectious diseases to non-communicable diseases including cancer [1]. The rising cancer burden in sub-Saharan Africa underlies the important role of anthracycline, which is still the chemotherapeutic drug class of choice for treating many cancers in the developing world [2]. In the Uganda Cancer Institute, more than half of the patients need anthracycline therapy [3].

Anthracyclines are a group of highly effective antineoplastic agents. Anthracycline therapy-related cardiac dysfunction (ATRC) is the well-recognized and noxious cardiovascular side effect from this chemotherapy [4]. Proposed risk factors of ATRCD include patient-related risk factors such as increasing age, gender, Black race, post-menopausal status and pre-existing cardiac risk factors (e.g., hypertension, diabetes mellitus, smoking and coronary artery disease) [5–8]. Other risk factors of ATRCD are therapy-related, including use of combination cancer therapy (e.g., trastuzumab), addition of mediastinal irradiation and higher doses of anthracycline [5,9].

## Research approvals



June 12, 2018

Dr. Zhanga Wau Zhu  
Uganda Heart Institute

Category of Review  
 Initial review  
 Continuing review  
 Amendment  
 Termination of study  
 SAEs

Dear Dr. Zhang,

Re: Approval of proposal #REC REF 2018-081

**"Detecting and treating sub-clinical Anthracycline therapy related cardiac dysfunction in low income country"**

Thank you for submitting an application for approval of the above – referenced **proposal**. The committee reviewed it and granted approval for one year, effective June 12<sup>th</sup>, 2018. Approval will expire on June 11<sup>th</sup>, 2019.

### Continuing Review

In order to continue work on this study (including data analysis) beyond the expiration date, the School of Medicine Research and Ethics Committee must reapprove the protocol after conducting a substantive, meaningful, continuing review. This means that you must submit a continuing report form as a request for continuing review. To best avoid a lapse, you should submit the request six (6) weeks before the lapse date. Please use the forms supplied by our office.

### Amendments

During the approval period, if you propose any change to the protocol such as its funding source, recruiting materials, or consent documents, you must seek School of Medicine Research and Ethics Committee approval before implementing it.

Please summarize the proposed change and the rationale for it in a letter to the School of Medicine Research and Ethics Committee. In addition, submit three (3) copies of an updated version of your original protocol application- one showing all proposed changes in bold or 'track changes,' and the other without bold or track changes.

Page 1 of 2

### Reporting

Other events which must be reported promptly in writing to the School of Medicine Research and Ethics Committee include:

Suspension or termination of the protocol by you or the grantor  
Unexpected problems involving risk to participants or others

Adverse events, including unanticipated or anticipated but severe physical harm to participants.

Do not hesitate to contact us if you have any questions. Thank you for your cooperation and commitment to the protection of human subjects in research.

Documents approved for use along with protocol:

- Both English and Translated Informed consent forms
- Data Collection tools

### Please Note:

1. Final approval is to be granted by Uganda National Council for Science and Technology.
2. All the study documents including the revised proposal, Informed Consent Forms, Data Collection Tools and any other study documents should be stamped by the REC.
3. Approval from National Drug Authority should be sought where applicable.
4. Approval for use of research devices should be sought from the Ministry of Health/Uganda National Council for Science and Technology where applicable.
5. Administrative clearance should be sought from the various study sites.
6. This study will be monitored by the School of Medicine Research Ethics Committee to assess for compliance to both national and international research guidelines for protecting the rights and welfare of research participants.
7. The REC should be involved in the dissemination of study findings to the different stakeholders. (This applies to staff research, collaborative research and PhD students).
8. The REC should be informed and involved in the process of study closure.

Yours sincerely,



Assoc. Prof. Joan Kalyango

Vice Chairperson School of Medicine Research and Ethics Committee



## Study Approval - (HS220ES)

Research Management - UNCST <research@uncst.go.ug>

周四 2018/9/6 14:39

收件人: Wan Zhu <raniox@hotmail.com>;

抄送: Zhang <zhangwanzhu2012@gmail.com>;



**Uganda National Council for Science and Technology**

*(Established by Act of Parliament of the Republic of Uganda)*

Dear Wan Zhu Zhang ,

I am pleased to inform you that on **06/09/2018**, the Uganda National Council for Science and Technology (UNCST) approved your study titled, **Detecting and treating subclinical anthracycline therapy related cardiac dysfunction in low income country**. The Approval is valid for the period of **06/09/2018** to **06/09/2020**.

Your study reference number is **HS220ES**. Please, cite this number in all your future correspondences with UNCST in respect of the above study.

Please, note that as Principal Investigator, you are responsible for:

1. Keeping all co-investigators informed about the status of the study.
2. Submitting any changes, amendments, and addenda to the study protocol or the consent form, where applicable, to the designated local Research Ethics Committee (REC) or Lead Agency, where applicable, for re-review and approval prior to the activation of the changes.
3. Notifying UNCST about the REC or lead agency approved changes, where applicable, within five working days.
4. For clinical trials, reporting all serious adverse events promptly to the designated local REC for review with copies to the National Drug Authority.
5. Promptly reporting any unanticipated problems involving risks to study subjects/participants to the UNCST.
6. Providing any new information which could change the risk/benefit ratio of the study to the UNCST for review.
7. Submitting annual progress reports electronically to UNCST. Failure to do so may result in termination of the research project.

Please, note that this approval includes all study related tools submitted as part of the application.

Yours sincerely,

Hellen Opolot

For: Executive Secretary

**UGANDA NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY**



## Uganda Cancer Institute

Upper Mulago Road P.O. Box 3935 , Kampala - Uganda. Tel: + 256 414 540 410 Fax : +256 414 530 729 Website: uci.or.ug

**Ref: REO/AC/02**

11<sup>th</sup> October 2018

To: Dr.Zhang Wan Zhu,  
Uganda Heart Institute/ Department of medicine -Mulago Hospital, Kampala.

**RE: Permission to Conduct Research at Uganda Cancer Institute - (ER: 015)**

Reference is made to your submission of the protocol entitled "**Detecting and Treating Subclinical Anthracycline Therapy Related Cardiac Dysfunction in Low Income Country (SATRACD Study)**" indicating your intent to conduct study participant recruitment form the Uganda Cancer Institute (UCI). We thank you for choosing Uganda Cancer Institute for this study implementation. Permission to conduct the research at UCI was granted. Please take note of the following issues as you conduct research at UCI:

- i) You and other study staff will be governed by the rules that govern the conduct and discipline of Public Officers.
- ii) Abide by the National Council for Science and Technology (UNCST) regulations for conducting research involving human participants and all relevant regulations.
- iii) Provide a copy of your research assistant training on human subject protection to the Research and Ethics Review Office before you begin data collection.
- iv) Ethical code and participant confidentiality are very pertinent so should be maintained at all times.
- v) Ensure that your research assistants are well trained on the study. Your contact person or Supervisor at UCI is Sr. **Nankoma Janet** at **0752935529** and **Dr. Nixon Niyonzima** at **0755677395** you are expected to work closely with them throughout your conduct of research at UCI.
- vi) You are requested to make a presentation about your research to the UCI staff
- vii) Provide a copy of your report at the end of the study to the Research Ethics Review office at UCI.

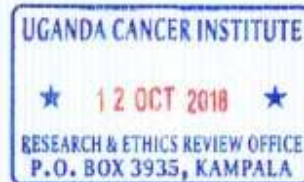
 1

This offer can be terminated in case you do not adhere to research ethics during your study conduct. By copy of this letter, the UCI Clinical head, and the assigned contact persons are informed about your study and strongly urged to act in case of any observed malpractices during your research conduct at the UCI.

Sincerely,



Dr. Nixon Niyonzima  
Head of Research and Training, Uganda Cancer Institute



C.C. Executive Director Uganda Cancer Institute

- “ The Hospital Administrator, U.C.I.
- “ UCI Clinical Head
- “ Ms. Nankoma Janet (Supervisor).



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



Room G50- Old Main Building  
Groote Schuur Hospital  
Observatory 7925  
Telephone [021] 406 6492  
Email: [hrec-enquiries@uct.ac.za](mailto:hrec-enquiries@uct.ac.za)  
Website: [www.health.uct.ac.za/fhs/research/humanethics/forms](http://www.health.uct.ac.za/fhs/research/humanethics/forms)

08 May 2020

**HREC REF: 054/2020**

**Prof K Sliwa**

Hatter Institute for Cardiovascular Research  
Room 412, Chris Barnard Building -FHS  
Email: [karen.sliwe@uct.ac.za](mailto:karen.sliwe@uct.ac.za)  
Student: [zhangwanzhu2012@gmail.com](mailto:zhangwanzhu2012@gmail.com)

Dear Prof Sliwa

**PROJECT TITLE: DETECTING AND TREATING SUBCLINICAL ANTHRACYCLINE THERAPY RELATED CARDIAC DYSFUNCTION IN LOW INCOME COUNTRY (PhD candidate Dr Zhang Wan Zhu)**

Thank you for your response letter dated 12 April 2020, addressing the issues raised by the Faculty of Health Sciences Human Research Ethics Committee (HREC).

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

**This approval is subject to strict adherence to the HREC recommendations regarding research involving human participants during COVID -19, dated 17 March 2020.**

**Approval is granted for one year until the 30 May 2021.**

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.

(Forms can be found on our website: [www.health.uct.ac.za/fhs/research/humanethics/forms](http://www.health.uct.ac.za/fhs/research/humanethics/forms))

**The HREC acknowledge that the student: - Dr Zhang Wan Zhu will also be involved in this study.**

**Please quote the HREC REF in all your correspondence.**

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate Institutional approval, where necessary, before the research may occur.

HREC 054/2020sa

Yours sincerely



**PROFESSOR M. BLOCKMAN**  
**CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE**

Federal Wide Assurance Number: FWA00001637.  
Institutional Review Board (IRB) number: IRB00001938  
NHREC-registration number: REC-210208-007

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

HREC 054/2020sa



## Uganda National Council for Science and Technology

(Established by Act of Parliament of the Republic of Uganda)

Our Ref: HS 220ES

31<sup>st</sup> October 2019

Dr. Zhang Wan Zhu  
Principal investigator  
C/o Uganda Heart Institute  
**KAMPALA**

**RE: APPROVAL OF TRANSFER OF SAMPLES FROM UGANDA CANCER INSTITUTE-UGANDA TO HATTER INSTITUTE FOR CARDIOVASCULAR DISEASE IN AFRICA, UNIVERSITY OF CAPE TOWN-SOUTH AFRICA.**

We refer to the request for approval of the Material Transfer Agreement (MTA) for the transfer of samples obtained in the research project titled, *'Detecting and treating subclinical anthracycline therapy related cardiac dysfunction in low income country'*

The UNCST on 25/10/2019 approved the transfer 600 serum and plasma samples through Entebbe International Airport to Hatter Institute for Cardiovascular Disease in Africa, University of Cape Town-South Africa for research purposes.

The approval is subject to the terms and conditions of the Material Transfer Agreement between Uganda Cancer Institute-Uganda and Hatter Institute for Cardiovascular Disease in Africa, University of Cape Town-South Africa. The Institutions should observe the conditions set by the Uganda-National Guidelines for Research Involving Humans as Research Participants on the use of the human materials.

The Commissioner Customs, Uganda Revenue Authority is duly informed by copy of this letter and is kindly requested to give you the necessary assistance to facilitate the transfer of the specimens within the terms of this agreement.

Yours sincerely,

Beth Mutumba  
**FOR: EXECUTIVE SECRETARY**

**Copied to:** The Secretary, Office of the President  
The Commissioner Customs, Uganda Revenue Authority

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### LOCATION/CORRESPONDENCE

Plot 6 Kimera Road, Ntinda  
P.O.Box 6884  
KAMPALA, UGANDA

### COMMUNICATION

TEL: (256) 414 705500  
FAX: (256) 414-234579  
EMAIL: [info@uncst.go.ug](mailto:info@uncst.go.ug)  
WEBSITE: <http://www.uncst.go.ug>