Peripheral blood flow in the carotid artery at rest to investigate cardiovascular health in breast cancer survivors: A pilot study.

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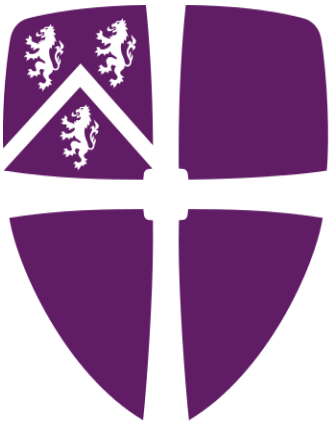

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Title: Peripheral blood flow in the carotid artery at rest to investigate cardiovascular health in breast cancer survivors: A pilot study.

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Abstract

Aim: Cardiotoxicity, damage to the heart as a result of cancer treatment, poses health risks for breast cancer survivors. The aim of this study was to investigate heart health in breast cancer survivors by assessing markers of heart health by using ultrasound on the common carotid artery in breast cancer survivors. The thesis is comparing differences in carotid artery blood flow parameters between breast cancer survivors and controls.

Methods: Differences in common carotid artery blood flow were investigated at rest between (n = 10) young healthy females (18-30 years), (n = 10) older healthy females (50 years and above) and (n = 10) breast cancer survivors (>18 years old) who have been treated with chemotherapy, radiation and hormonal treatment. Participants were recruited via criterion-based purposive sampling. Height (cm), weight (kg), were collected and used to calculate BMI (kg/cm^2). Heart rate (bpm) and blood pressure (bp) were also collected. Intima media wall thickness (IMT) of the common carotid artery (CCA), diameter of the CCA in transverse and longitudinal view, peak systolic velocity (PSV) and end-diastolic velocity (EDV) were also taken via ultrasound using standard procedures.

Results: Data suggests that breast cancer survivors (73.61 cm/s) have lower PSV when compared to young healthy females (95.7 cm/s), (P = 0.006). Breast cancer survivors (0.05 cm) have a higher IMT when compared to young healthy females (0.03 cm), (P = 0.001). Older healthy females (0.05 cm) also had a higher IMT compared to young healthy females (P = 0.001), but no differences were observed between older healthy females and breast

cancer survivors (P = 0.644). No statistical significance in diameter of the CCA longitudinal and transverse were observed between groups.

Conclusion: Analysis suggests a reduction in PSV in breast cancer survivors compared to young healthy females. The increased IMT may be due to cancer treatment in breast cancer survivors. However, whether these differences are due to age or cancer is unclear at this time – more studies investigating this is needed for additional insight.

Chapter 1 – Introduction

1.1. Introduction and Background

Cardiotoxicity is a pressing issue within the cardio-oncology sector, as many as 30 % of cancer patients may experience cardiotoxicity during or after cancer treatment has finished (Herrmann, 2020). Cardiotoxicity is defined as a decrease in the heart function due to chemotherapy or more recently due to other cancer treatments such as radiotherapy and hormonal treatments (Yeh *et al.,* 2014). This means that the heart pumps blood around the body less efficiently than normal. Cardiotoxicity damages the myocardium which reduces blood flow from the left ventricle (Wang *et al.,* 2021). It is usually described by one or more of these following symptoms, reduction in left ventricular ejection fraction (LVEF), heart failure symptoms such as chest pain, shortness of breath, fluid filled lungs and cardiomyopathy. Cardiomyopathy is caused when there are alterations in gene expression, cell morphology changes, and shifts in metabolism inside the cardiac myocytes (Harvey and Leinwand, 2011). Consequentially leading to heart failure. Heart failure symptoms include reduced ejection fraction, fatigue and cardiac ischemia (Brieler *et al.,* 2017; Yeh *et al.,* 2014).

Cardiotoxicity usually occurs soon after cancer treatment and can increase the patients' risk of cardiovascular disease in survivorship (Cardinale *et al.,* 2020; Rosa *et al.,* 2016; Shaikh and Shih, 2012). Research demonstrates a strong link between cancer survivorship and cardiovascular disease (Choi *et al.,* 2023; Reding *et al.,* 2022; Mehta *et al.,* 2018; Gulati and Mulvagh, 2018; Xie *et al.,* 2015). For patients who already have cardiovascular disease prior to starting any cancer treatment, cardiotoxicity may be more significant due to the cardiovascular system already being under stress from the cardiovascular disease itself (Rosa *et al.,* 2016; Shaikh and Shih, 2012).

Three mechanisms are thought to lead to cardiotoxicity, including oxidative stress, the production of free radicals and hypoxia (Singh *et al.,* 2023). Oxidative stress is caused by an imbalance between oxidants and antioxidants (more so heavily containing oxidants), which then leads to a disruption of redox signalling, molecular and control damage (Sies *et al.,* 2017). Free radicals are reactive chemical species that have a single unpaired electron in its outer orbit, and they cause damage to healthy cells by altering their structure due to the free electron (Matthew *et al.,* 2011). Lastly, hypoxia is when there is an interference with energy metabolism and an increased demand of oxygen. This causes damage to the heart due to the starvation of oxygen to the heart muscle (Iqubal *et al.,* 2018). These conditions are induced by certain drugs and exposure to radiation (Singh *et al.,* 2023).

For many decades cardiotoxicity was always associated with anthracyclines (ANTHS); however, there is evidence to suggest that more anti-cancer drugs may be associated with cardiotoxicity Cardiotoxic drugs now include anthracyclines, amphetamines, mitomycin, paclitaxel and zidovudine with anthracyclines being the most common and causing the most severe cases of cardiotoxicity (Iqubal *et al.,* 2018; Rochette *et al.,* 2015). Drug treatments that are used currently to treat patients with cancer include anthracyclines, human epidermal growth factor receptor 2 (HER2+) inhibitors, Taxanes, alkylating agents and antimetabolites (Cancer Research UK, 2023).

There is also evidence to suggest that radiotherapy can also cause cardiotoxicity (Koutroumpakis *et al.,* 2020). The radiation delivered to the body via radiotherapy can then cause direct damage to the heart. This is most pronounced, the radiotherapy treatment being directed towards the heart, such as when targeting the cancer in the breast. It is estimated that breast cancer patients who have received radiotherapy to the breast have a 0.5 % - 3.5 % increased lifetime risk of cardiovascular events such as a myocardial infarction, with the risk being highest in those that have had radiation to the left breast (Brenner *et al.,* 2014). Any

cancer survivor can be affected by cardiotoxicity; however, it is of particular concern in breast cancer survivors. In breast cancer patients that have radiotherapy, the radiation is directed towards the chest area to target the breast cancer. If the cancer is in the left breast it can lead to more negative implications due to the hearts positioning leaning more towards the left side (Brenner *et al.,* 2014).

Existing guideline protocols for assessing cardiotoxicity include left ventricular ejection fraction (LVEF), echocardiography and blood biomarkers (Raschi *et al.,* 2017; Dolci *et al.,* 2008; McGowan and Cleland, 2003). At present, there is no gold standard assessment of measuring cardiotoxicity (Jurcut *et al.,* 2008) and it is assessed through a variety of different markers. This may vary depending on the type of chemotherapy used and the physician's preferred method. Currently methods that are used to identify ANTHS cardiotoxicity include electrocardiograms (ECG), biochemical markers, functional measurement of the heart, and morphologic assessment (Hrdina *et al.,* 2000).

Though it has not been explored in research before, blood flow may be a key factor in cardiotoxicity. Due to the heart being a less efficient pump, less blood is able to be pumped out with each stroke. It would be expected that there would be a notable reduced difference in more common blood flow markers such as peak systolic velocity (PSV). The left common carotid artery (CCA) is the main artery that carries oxygenated blood from the heart to the brain, arising directly from the aorta (Chandra *et al.,* 2017). The carotid artery is an accessible part of the body and blood flow that arises from the heart (Yeh *et al.,* 2014). It is anticipated that changes in blood flow could be measured in this vessel.

Traditionally, blood flow in cardiotoxicity is measured through left ventricular ejection fraction, and this method cannot detect cardiotoxicity until it is advanced and severe (Raschi *et al.,* 2017). Additionally, a stable LVEF value does not mean that there is no cardiac injury

or cardiotoxicity (Raschi *et al.,* 2017). Ultrasound may prove to be a valuable method of measurement for assessing cardiotoxicity though measurements of blood flow. It is cost effective, safe, accessible, widely available, side effect free, radiation free, and it does not require many hours of training (Phenix *et al.,* 2014). Whilst the use of ultrasound on the carotid artery to investigate cardiotoxicity has never been explored before it may prove to be very a valuable method into finding ways of predicting and measuring cardiotoxicity.

1.2. Study Rationale

Understanding alteration in carotid artery blood flow in breast cancer survivors may help explain the interrelationship between cardiotoxicity and CVD. However, the methods of measuring these features are largely unexamined. This may be due to the fact that currently there is no standard procedure of measuring or monitoring cardiotoxicity that is governed by any clinical or practical guidelines. Blood flow is thought to be linked to cardiotoxicity as heart function is reduced, meaning that blood flow is less efficient when being pumped from the heart (Yeh *et al.,* 2014). Using ultrasound as a measure to detect cardiotoxicity may help oncologists, cardiologists and cardio-oncologists monitor heart health quickly so in turn cancer treatment doses can be altered easily if required, if the function of the heart is reduced. The ability to use ultrasound as a measure of cardiotoxicity would mean that the method would be more accessible and more widely available compared to other methods which are currently used.

Conducting this research is important because it holds the potential to generate novel and innovative insights into methods to potentially identify and predict cardiotoxicity. Furthermore, little is known about the early changes in blood flow that may indicate cardiotoxicity. The measurement of PSV in particular, may be a potential early marker of

cardiotoxicity, as PSV is the highest velocity of blood that the heart pumps during systole (Mari *et al.,* 2005). Changes in PSV may occur prior to other cardiotoxicity symptoms.

1.3. Study Aims

The aim of the current study is to investigate CCA ultrasounds' ability to detect differences in blood flow and physical characteristics in the carotid artery at rest between young healthy females, older healthy females and breast cancer survivors who have been treated by chemotherapy, radiation and/or hormonal treatments.

1.4. Study Objectives

The study objectives are:

1. To investigate whether it is feasible to use carotid artery ultrasound to detect differences in blood flow and physical characteristics in breast cancer survivors as a potential metric of cardiotoxicity.
2. To identify if there is a difference in blood flow and physical characteristics in the carotid artery between the young healthy females, older healthy females and breast cancer survivors using carotid artery ultrasound.
3. To identify the degree of difference there is in blood flow and physical characteristics at rest between the young healthy females, older healthy females, and breast cancer survivors by comparing the values that are collected between each group.

Chapter 2 - Literature Review

2.1. Cardiotoxicity

Cardiotoxicity, as a unifying definition, is understood to be a decline in cardiac function due to cancer treatment (Herrmann, 2020). Despite the known effects of cancer treatments on the heart there is no universal definition or clinical list of symptoms and signs to detect cardiotoxicity officially (Wickramasinghe *et al.,* 2016). Cancer treatments are linked to many cardiovascular issues such as severe arrhythmia, ischemia, infarction, and damage to cardiac valves as well as the conduction system of the heart (Ewer and Ewer, 2015). This lack of a universal definition can cause issues within the field as different studies may use different definitions. Therefore, more research is needed into the characterisation, monitoring and eventually prevention of these negative cardiovascular effects prior, during and after cancer treatment has finished to then prolong the lives of cancer survivors.

Cardiotoxicity can be classed into types: type 1 and type 2 cardiotoxicity. Type 1 is categorised through injury to the heart tissue which is then less likely to be reversible whereas type 2 cardiotoxicity is categorised as cardiac dysfunction which is more likely to be reversible after treatment has ended (Wickramasinghe *et al.,* 2016). Breast cancer survivors may experience both type 1 and type 2 cardiotoxicity; however, like with an overarching definition of cardiotoxicity, these categorisations are not officially recognised or considered universal.

In addition to cardiotoxicity, pre-existing cardiovascular risk factors such as hypertension and genetics are also considered as strong predictors for whether a patient will develop cardiovascular disease after cancer treatment has ended (Wickramasinghe *et al.,* 2016). Cardiotoxicity is strongly associated with cardiovascular disease, which can be temporary or permanent. Cardiotoxicity has a high chance of turning into cardiovascular disease due to the

damage that the cancer treatment causes to the heart. It is not known exactly what mechanism causes cardiotoxicity however it is often thought to be a combination of factors such as oxidative stress, damage to DNA, cellular senescence, cardiomyocyte death, cardiac progenitor cell death, cardiac fibroblasts death, and endothelial cell death (Schettini *et al.,* 2021). These mechanisms will be explored next in this review.

2.2. Mechanisms of Cardiotoxicity

Cancer treatments today can cause an increase in oxidative stress, progenitor cell inhibition, titin proteolysis, neuregulin/ErbB inhibition and apoptosis (Hahn *et al.,* 2014). All of these mechanisms can then cause cardiotoxicity by causing intracellular damage (Moudgil and Yeh, 2016; Hahn *et al.,* 2014). Progenitor cells are the main cells that are involved with cardiac regeneration and are activated after there is injury to the heart. These cells contribute to the renewal of cardiomyocytes (Bryl *et al.,* 2024). Progenitor cell inhibition is therefore the cessation in production of these cells. The cessation in progenitor cells production leads to cardiotoxicity due to the cardiomyocytes not being able to renew or regenerate for normal cardiac function.

Titin is a sarcomere protein that determines the stiffness and ventricular distensibility of cardiomyocytes (Müller *et al.,* 2021). Titin is broken down through the process of 'proteolysis' (Müller *et al.,* 2021). Titin proteolysis leads to cardiotoxicity due to the cardiomyocytes becoming stiff, making it harder for the heart to pump blood around the body.

Neuregulin-1 is a cardiomyocyte proliferator, and it plays a key role in the growth of the heart, proliferation, differentiation and apoptosis (Wang *et al.,* 2022). Neuregulin/ErbB inhibition then inhibits all of these mechanisms from occurring as normal (Wang *et al.,* 2022). Neuregulin/ErbB inhibition leads to cardiotoxicity due to the reduction of neuregulin-1 in the

cells. This reduces the cell's ability to proliferate and regenerate injured myocardial tissue (Ponnusamy *et al.,* 2017).

2.3. Fundamentals of Breast Cancer

Breast cancer is a cancer found in breast tissue (Figure 1). It is one of the most diagnosed cancers in women, however, survival rates have significantly improved due to wider availability of treatment options and the advancement of healthcare (Sharma *et al.,* 2010). Breast cancer is known as a heterogenous disease in which it can be classed as either one of four subtypes, which include luminal A, luminal B, HER2 and triple-negative breast cancer (Burguin *et al.,* 2021). The classification of breast cancer into one of four subtypes is important because it gives prognostic information regarding the tumour and can help identify the best treatment option to eliminate the breast cancer tumour (Sims *et al.,* 2007).

The breast is composed of glandular tissues and stromal tissues and the exact precise mechanisms in how breast cancer starts are unclear, however it is thought to be linked to genetic and environmental factors (Barzaman *et al.,* 2020; Sharma *et al.,* 2010). Additionally, cancer cells are the result of a mutation of DNA and/or RNA. The mutations can be random and sporadic, or they can also be induced by factors such as radiation, viruses, bacteria, heat, fungi, parasites, chemicals in the air, food, water, genetic predispositions, free radicals, ageing of DNA and lastly RNA (Sharma *et al.,* 2010). Cancerous cells have a disruption in their division process, which results in cell proliferation and tumour growth (see figure 2). Furthermore, breast cancer stem cells are the main component in the aggressiveness of different tumours (Barzaman *et al.,* 2020). This is due to the breast cancer stem cells having self-renewal and differentiation properties, which contribute to the aggressiveness of the tumours (Song and Farzaneh, 2021).

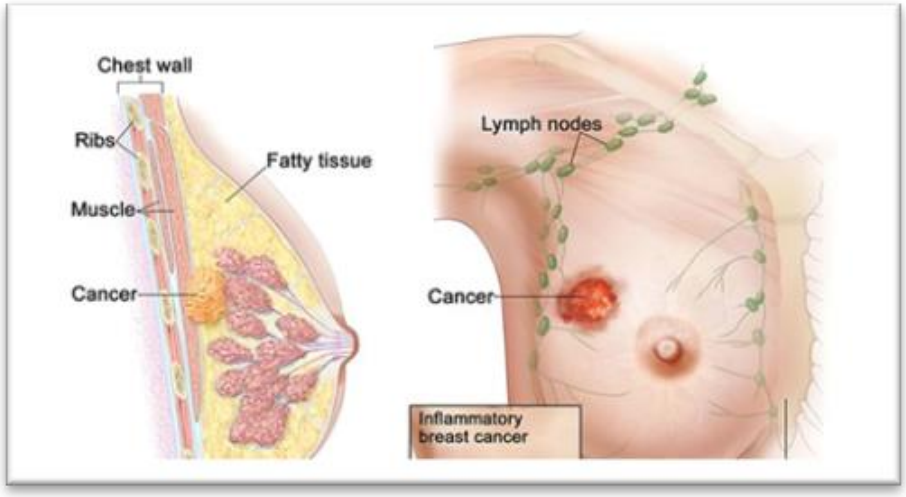

Figure 1*:* Displaying where breast cancer is located and that it can be found in the fatty tissue of the breast as well as growing into the muscle and chest wall. Image from (Almurshidi and Abu-Naser, 2018).

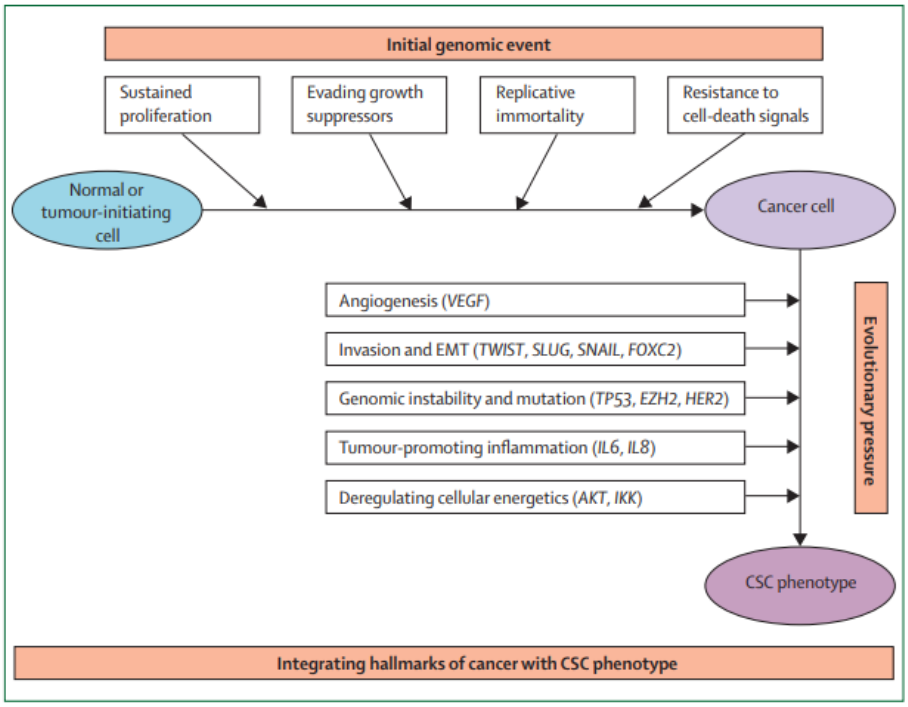

Figure 2: Displaying how healthy cells can turn into cancerous cells, which then lead to tumours. Image from *(Badve and Nakshatri, 2012)*.

Risk factors for breast cancer include gender, age, late age of menopause, hormonal contraception, genetic factors, being overweight, alcohol consumption, diet and smoking as well as exposure to radiation and having diabetes (Momenimovahed and Salehiniya, 2019). Despite improvements in treatment, incidence rates of breast cancer remain high worldwide (Arnold *et al.,* 2022). There were over 2.3 million cases of breast cancer, and 685,000 deaths were reported in 2020. Globally there is a large variation across countries and regions, as incidence rates range from < 40 per 100,000 females in Asian and African countries to over 80 per 100,000 in New Zealand, Northern America, Australia, and parts of Europe. Countries that are transitioning have a disproportionate number of breast cancer deaths when compared with transitioned countries (Arnold *et al.,* 2022).

2.4. Treatments for Breast Cancer and the Effects they have on the Cardiovascular System

There are a wide range of different treatments available for the treatment of breast cancer with all of them having some effect on the cardiovascular system, although some drugs having more adverse effects to the cardiovascular system than others (Brana and Tabernero, 2010). There is increasing research interest into the area of cardiotoxicity especially looking into cancer management, which include the types of treatments/drugs that are offered. This is due to the risk and consequential adverse effects after cancer treatment such as ANTHS and TRZ leading to cardiotoxicity and CVD (Brana and Tabernero, 2010). The type of treatment offered to cancer patients depends on where the cancer is located, the age of the patient, the patient's performance status, if the patient has any comorbidities and lastly preference of the patient (Jerusalem *et al.,* 2019). If the cancer patient has any comorbidities relating to the cardiovascular system, then this can put the cancer patient at further risk of developing

cardiotoxicity. The treatments that will be discussed in the following section are chemotherapy drugs, radiotherapy, and hormone therapy (Cancer Research UK, 2023).

2.5. Treatments Used in Breast Cancer

1. Chemotherapy

Chemotherapy is a treatment commonly used in breast cancer patients to try and eradicate the cancer in the breast tissue (Behranvand *et al.,* 2021). Chemotherapy drugs destroy the tumour cells via various mechanisms. These include starting internal and external apoptosis, cell cycle arrest, alkylation of DNA which causes a damage to the DNA leading to cell death. Mechanisms also include ceasing DNA or RNA synthesis, interfering with the microtubules, causing damage directly or indirectly to the tumour cells through the production of reactive oxygen species and the prevention of topoisomerases which stops cell growth via cell proliferation (Behranvand *et al.,* 2021). Most of these mechanisms listed above cause oxidative stress which leads to the damage of the tumour cells (Behranvand *et al.,* 2021).

Moreover, there are chemotherapy specific mechanisms that can also occur which can also lead to cardiotoxicity. Sandoo and colleagues (2014) suggest ANTHS cause the HER2 survival pathways to be activated to then counteract the stress to the cardiomyocytes from the ANTHS treatment. HER2 survival pathways are the main mechanism process that allows cell proliferation to occur (Gutierrez and Schiff, 2011). Additionally, when patients are treated with Trastuzumab (TRZ), another type of chemotherapy, after being treated with ANTHS, this blocks the survival pathways causing more stress on the cardiac tissue consequently affecting cardiomyocytes and leading to cardiac heart failure (Sandoo *et al.,* 2014). In patients that have been treated with TRZ it has been found that the changes in the

myocardium are not related to the dose of TRZ. TRZ is thought to also cause negative effects on the coronary and peripheral vasculature rather than just on cardiomyocytes, consequently increasing the risk of cardiac heart failure (Sandoo *et al.,* 2014). This may explain why the dose-response relationship between TRZ, and cardiotoxicity is less pronounced. Additional chemotherapy drugs and their mechanism of action are summarised in Table 1.

2. Radiotherapy

Radiotherapy is a non-surgical method which uses radiation to puncture the DNA in the cancer cells. This then prevents their growth and division in order to eradicate them (Mohan *et al.,* 2019). The dosage of radiation given to each patient can vary depending on the tumour size, location, and the stage of cancer (Mohan *et al.,* 2019). Approximately 50 % of cancer patients have radiotherapy at some point in their cancer treatment. Radiotherapy for breast cancer includes both types of radiotherapy including external beam radiation therapy (EBRT) and internal beam radiation therapy (IBRT) also known as brachytherapy (Mohan *et al.,* 2019). In EBRT the radiation (X-rays) is directed to the surface of the cancer, whereas in IBRT a radioactive implant is put near the tumour (Mohan *et al.,* 2019). This is a problem for cardiovascular health as it can damage the heart, especially if the tumour is in the left breast. This is due to the hearts positioning being more towards the left side of the chest.

3. Hormone Treatments – Aromatase Inhibitor (AI)

Hormone treatments such as anastrozole, letrozole and exemestane are very commonly used in breast cancer patients, with letrozole being used the most out of the three

(Mukherjee *et al.,* 2022 ; Dutta and Pant, 2007). Letrozole is a powerful hormone medication that works by targeting and inhibiting the aromatase, also known as the aromatase inhibitor (AI). It helps in the treatment of HR + breast cancer because it can boost the production of the follicle stimulating hormone. Letrozole also causes apoptosis, necrosis, fibrosis, and necrosis which all cause damage to cancer cells (Mukherjee *et al.,* 2022).

However, Letrozole can cause an increased risk of cardiovascular disease, this is due to the reduced oestrogen caused by the letrozole (Mukherjee *et al.,* 2022). Letrozole is typically administered to breast cancer patients in doses between 0.5 – 2.5 mg, in which they are taken daily to supress oestrogen levels in the blood. The reduction of oestrogen levels in the blood then leads to a significant increase in serum lipid profiles such as total cholesterol levels in breast cancer patients hence increasing the risk of cardiovascular disease (Mukherjee *et al.,* 2022). The other aromatase inhibitors, such as anastrozole and exemestane, work through the same mechanisms as letrozole, however these are less commonly used (Geisler, 2011).

Table 1: Chemotherapy Drugs Used to Treat Breast Cancer		
Drug Class	**Drug**	**Role in Cardiotoxicity and Mechanism of Action**
Alkylating Agents	Carboplatin	• Can cause tissue injury and reactive oxygen species. • Causes oxidative stress associated cardiac dysfunction (Cheng *et al.,* 2008). • Associated with cardiotoxicity more frequently if the individual had a history of cardiovascular disease prior to treatment (Bursac *et al.,* 2016). • Carboplatin causes the alkylation of DNA which can then lead to cell death in the tumour cells (Behranvand *et al.,* 2021).
Antimetabolites	Capecitabine	• Symptoms can include: changes in ST-T waves on ECG measurements, myocardial infarction, dysrhythmias, pulmonary edema (Molteni *et al.,* 2010). • Severe cardiotoxicity can occur, and even in young and low risk patients with no cardiovascular risk factors (Molteni *et al.,* 2010).

Anthracyclines and Alkylating Agents	Epirubicin and Cyclophosphamide (EC) as a Combination Treatment	• Cyclophosphamide can cause heart failure, neurohumoral activation and mitral regurgitation (Florescu *et al.,* 2013). • Cyclophosphamide prevents cell division by crosslinking the DNA strands (Behranvand *et al.,* 2021).
Anthracyclines and Alkylating Agents	Doxorubicin and Cyclophosphamide (AC) as a Combination Treatment	• Has severe adverse side effects on cardiomyocytes – can lead to cardiomyocyte death (Zhang *et al.,* 2021) . • Causes oxidative stress from an imbalance of reactive oxygen species and antioxidants (Zhang *et al.,* 2021).
Taxanes and Alkylating Agents	Docetaxel and Cyclophosphamide (TC) as a Combination Treatment	• Myalgia, arthralgia, edema, myocardial infarction, and febrile neutropenia are more common with this drug combination (Jones *et al.,* 2006).
Antineoplastics, Antineoplastics and Antimetabolites	Mitoxantrone, Mitomycin C and Methotrexate (MMM) as a Combination Treatment	• Thrombocytopenia, haematological toxicity (Jodrell *et al.,* 1991). • Lower incidence rates of severe cardiotoxicity (Pfeiffer *et al.,* 1992; Jodrell *et al.,* 1991). • MMM has a higher overall median survival rate of 16 months when compared to CMF of 12 months (Fukuda *et al.,* 2015).

		• Mitomycin C prevents the division of cells by DNA crosslinking and causing oxidative stress to the DNA which then causes DNA damage (Behranvand *et al.,* 2021).
Antimetabolites and Taxanes	Gemcitabine and Paclitaxel as a Combination Treatment	• Hematologic toxicity, especially neutropenia (Aogi *et al.,* 2010; Albain *et al.,* 2008). • Neuropathy and fatigue (Albain *et al.,* 2008).
Anthracyclines	(Epirubicin, Eribulin, Doxorubicin).	• Among the more aggressive chemotherapy drugs used to treat breast cancer. • Higher risk of developing cardiotoxicity which subsequently can lead to congestive heart failure (Schettini *et al.,* 2021). • Can cause left ventricular dysfunction, heart failure, myocarditis and arrythmia (Florescu *et al.,* 2013). • Doxorubicin inhibits topoisomerase-II-mediated repair and produces free radicals (Behranvand *et al.,* 2021).

HER2+ Inhibitors	Trastuzumab	• Has an association with an increased risk of developing cardiotoxicity, as in Jerusalem and colleagues (2019) work they found that 3 % to 7 % of patients who were treated with Trastuzumab experienced cardiac dysfunction. • It has been suggested that cardiomyocyte death results due to ErbB2 (HER2) blockade and the increased production of reactive oxygen species (Jerusalem *et al.*, 2019). • Often associated with heart conditions such as heart failure, left ventricular dysfunction and arrythmia (Florescu *et al.,* 2013). • Currently Trastuzumab (TRZ) is used as the first treatment option for individuals with metastatic HER2 + tumours. • Use of TRZ in cases is forced to be limited due to the risk of cardiotoxicity and cardiovascular dysfunction.
Taxanes	Paclitaxel, Docetaxel	• Both Paclitaxel and Docetaxel have very similar structures chemically. • Both bind to tubulin, promote stabilisation of microtubules and cause G2M cell cycle arrest (Saloustros et al., 2008).

		• Cardiac dysfunction has shown to be less frequent in patients treated with Docetaxel alone (Lyseng-Williamson and Fenton, 2005). • Neutropenia and febrile neutropenia were found to be common amongst patients treated with Docetaxel (Lyseng-Williamson and Fenton, 2005). • Paclitaxel can cause sinus bradycardia, ventricular tachycardia, heart failure, atrioventricular block, and ischemia (Florescu et al., 2013). • Paclitaxel stabilises microtubules and interferes with normal mitosis, and apoptotic cell death (Behranvand *et al.,* 2021).

2.5. Findings from Other Drugs Used to Treat Other Cancers

Nuver and colleagues (2005) investigated patients who had testicular cancer who were treated with cisplatin-based chemotherapy. They found that there was an increase in the thickness of the intima-media of the carotid artery. This change may mean that vascular damage has occurred due to the chemotherapy treatment, and it may be of prognostic significance for the development of any future cardiovascular complications.

2.6. Exercise and Cancer Treatment

Aerobic exercise has been suggested as a drug-free treatment which can reduce the effect of ANTHS (e.g., doxorubicin) induced cardiotoxicity (Scott *et al.,* 2011). Aerobic exercise is known to have positive effects on systolic and diastolic function and reduce the effect of cardiac remodelling. This then results in the heart being able to cope with exercise better in cancer survivors and cancer patients with heart failure. Scott and colleagues (2011) identified that overall aerobic exercise is a positive treatment in preventing and/or treating ANTHS induced heart injury/damage. This is also an accessible treatment for all current cancer patient and cancer survivors to be able to partake in to help strengthen their heart muscle. Exercise is also a useful tool in breast cancer survivors as in a systematic review conducted by Bekhet and colleagues (2019) there were strong links to exercise improving the quality of lives in breast cancer survivors. This in turn reducing the risk of cardiovascular disease and improving overall heart health.

2.7. The Interaction between Cardiovascular Disease and Breast Cancer

Breast cancer treatments that are used today can have a negative impact on cardiovascular health, including the accelerating of pre-existing cardiovascular disease. For women with pre-existing cardiovascular disease, this can then have an impact on the types of treatment given (Mehta *et al.,* 2018). The treatments that are used today can have an impact on the hearts ability to pump blood around the body, as described above. When that is combined with an individual who has pre-existing cardiovascular disease this can then cause serious implications for their heart health. This is also known as the “multiple-hit” hypothesis proposed by Jones and colleagues (2007) (see figure 3), which states that a large amount of early breast cancer patients at diagnosis already have pre-existing or at an increased risk of cardiovascular disease risk factors (Gulati and Mulvagh, 2018). This then increases the risk

of cardiovascular injury. These direct effects of cancer treatment then combine with pre-existing or increased risk of cardiovascular risk to the patient can exacerbate that pre-existing cardiovascular risk and increase the risk of fatality due to cardiovascular mortality (Gulati and Mulvagh, 2018).

Both cardiotoxicity and cardiovascular disease share similar mechanisms that result in both conditions negatively affecting the hearts' ability to pump blood around the body effectively. These mechanisms include oxidative stress, inflammation, and the death of cardiomyocytes (Hahn *et al.,* 2014; Elahi *et al.,* 2009). Breast cancer and cardiovascular disease share some common risk factors such as age, diet, family history, being a smoker, physical activity, obesity, and alcohol intake. Improvements in the prognosis of breast cancer has resulted in an increased number of breast cancer survivors, this then increases the risk of cardiotoxicity from treatments given (Mehta *et al.,* 2018). CVD has been found to be a greater risk to an older women's health when compared to breast cancer itself (Mehta *et al.,* 2018).

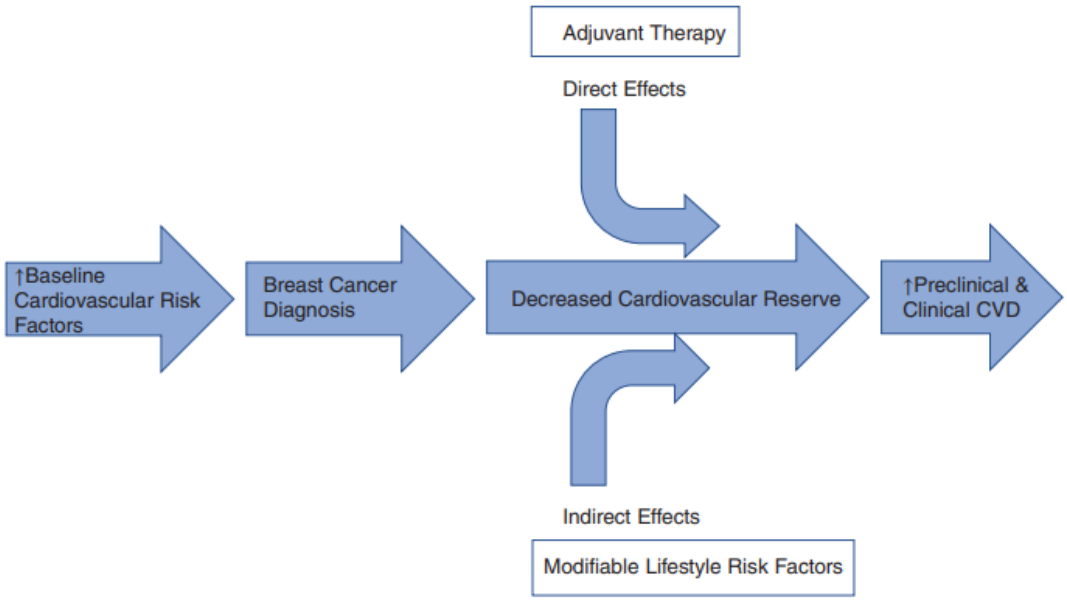

Figure 3. Visual representation of the "multiple-hit hypothesis" from (Jones *et al.,* 2007) theory. This shows that individuals who already have pre-existing cardiovascular disease or

are at an increased risk and that are diagnosed with cancer, have a decreased cardiovascular reserve which increases their risk of cardiovascular events and mortality from CVD. Image obtained from (Gulati and Mulvagh, 2018).

2.8. Methods in which Cardiotoxicity can be Measured

To date there is no standardised gold standard method to assess adult cancer patients or adult cancer survivors for cardiotoxicity, instead this is down to the doctor's discretion in which method they decide to use (Lyon *et al.,* 2022; Jurcut *et al.,* 2008). The 2022 ESC guidelines on cardio-oncology exist but there is lack of evidence in many of the suggested monitoring protocols and several protocols are based on clinical practice and not research studies (Lyon *et al.,* 2022). There are many different methods that doctors may use to decide to assess/monitor cancer patients and cancer survivors' cardiovascular system, some of these include Echocardiography, Left ventricular ejection fraction (LVEF), Biomarker test, Magnetic Resonance Imaging (MRI), Electrocardiogram (ECG) and 24-hour Holter monitoring (Afrin *et al.,* 2022; Stone *et al.,* 2021; Jurcut *et al.,* 2008; Galderisi et al., 2007).

Echocardiography

Echocardiography is a method in which an echocardiography machine connected to a transducer is used to look at the heart in real time and to take various images of the heart in different views and cardiac cycle stages (Lang *et al.,* 2015). It is the easiest method to access to monitor cardiotoxicity, with measurements being able to detect left ventricular ejection fraction (LVEF), the function of the valves and the walls of the heart, as well as being able to detect any abnormal heart rhythm (Florescu *et al.,* 2013). Although echocardiography is non-invasive and inexpensive there is limitations to this method. Firstly, it is down to the skill of the echocardiographer to gain clear good quality images, however this is not always possible

so this may lead to missing key factors and potential misdiagnoses (Florescu *et al.,* 2013). Secondly, it can also subjective, meaning different echocardiographers may interpret scan results differently and may not agree with all interpretations in a scanning report prepared by a colleague (McGowan and Cleland, 2003). This then poses the challenge of a medical professional missing results that imply that a patient has cardiotoxicity. This could have huge negative consequences on the patient's life after cancer treatment has finished, as early identification of cardiotoxicity an improve outcomes for patients and reduce complications such as a cardiac arrest.

Left Ventricular Ejection Fraction (LVEF)

LVEF measurements are either obtained by echocardiography machines, magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET) or multiple-gated acquisition (MUGA) (Foley *et al.,* 2012). The measured LVEF is then compared to normative values or compared to values obtained from a previous scan, if it is a follow up (e.g., cancer chemotherapy treatment) (Ewer and Lenihan, 2008). LVEF is a popular method which medical professionals use to assess the effects of cancer treatment, however there is much debate currently around whether this method alone is sufficient and reliable enough to make any clinical judgements (Posch *et al.,* 2022). This is due to LVEF not being the most suitable method to assess cardiac function reserve, and it's the subjectivity in echocardiography sector of the assessment, as described above.

Similarly, the use of LVEF alone is limited in both the diagnostic and prognostic ability (Yu and Ky, 2015). A drop in LVEF does not always indicate that there is actual cardiac injury (Raschi *et al.,* 2017). In addition, a stable LVEF value does not mean that there is no cardiotoxicity or cardiac injury in the patient (Raschi *et al.,* 2017). For the left ventricle to show a decrease in ejection fraction the myocardium will have been damaged quite severely,

therefore EF that has lowered considerably after treatment is a marker to show advanced myocyte damage. LVEF will not usually be able to detect cardiotoxicity in the early stages but only when it is in advanced stages. This suggests that it may not be suitable as a preventive marker of cardiotoxicity, but only as a marker of late-stage damage (Ewer and Lenihan, 2008). LVEF is also known to display a low predictive power in detecting myocardial injury (Horacek *et al.,* 2014).

Biomarker Test

Biomarker tests are becoming more popular, and significantly more research is being done into this area to understand their potential use to determine risk or diagnose cardiotoxicity (Tan and Lyon, 2018). Troponin and brain natriuretic peptides are the most well-known and used cardiac biomarkers to detect heart failure, a symptom of cardiotoxicity (Tan and Lyon, 2018). Cardiac troponin I and cardiac troponin T are both part of the contractile mechanisms located within the sarcomere and cytoplasm in cardiomyocytes. Cardiac troponins are proteins that are released into the blood when damage to the myocyte has occurred. Usually, the level of troponin will increase in the blood stream within 2 or 3 hours after damage to the myocyte has occurred (Curigliano *et al.,* 2016). Increases in cardiac troponins in the blood correlate with severity of cardiac injury (Horacek *et al.,* 2014). Various studies have shown that cardiac troponins may detect cardiotoxicity before there are any notable changes in LVEF in patients treated by chemotherapy (Curigliano *et al.,* 2016; Ky *et al.,* 2014; Lipshultz *et al.,* 1997).

Brain natriuretic peptides (BNP) are secreted by myocardial cells which are located on atria and ventricles. They are predominantly secreted by the left ventricle myocardial cells (Tsekoura *et al.,* 2003). Brain natriuretic peptides have been established as a heart failure biomarker (Fu *et al.,* 2018). High levels of brain natriuretic peptides indicate heart failure,

and low levels of BNP predict that there is a reduced risk of any major cardiovascular events (Porapakkham *et al.,* 2010).

However, there are still limitations to biomarker use. Biomarkers can have interactions with other factors such as drugs, which may increase or decrease the levels of a biomarker (Mayeux, 2004). Interindividual variability is also a concern from person to person, as an individual's diet varies from person to person, so the amount of biomarker detected in an individual may be higher or lower depending on what the person has eaten (Mayeux, 2004). Genetics can also influence how individuals metabolise different substances, so it is very important to consider these factors when looking at results of an individual's biomarkers. Laboratory errors are also a concern with biomarkers, as each lab may operate differently, intraindividual variability is one of the main concerns with errors from and within the laboratory (Mayeux, 2004). Biochemical markers can be useful in the detection of injury to the myocardium and as a predictor of ventricular dysfunction. However, there are no guidelines or cut off points as to how much of a certain biochemical marker in the blood is a problem. It is also unclear which biochemical marker is the most important when detecting cardiotoxicity (Dolci *et al.,* 2008). These factors make it difficult to solely use biomarkers to detect cardiotoxicity.

Cardiac Magnetic Resonance Imaging (CMR)

Cardiac magnetic resonance imaging is a safe and non-invasive method which can identify any anatomical and functional changes in the myocardium (Jafari *et al*, 2020). CMR does not use ionising radiation, so therefore it can be a popular choice for cancer patients who are receiving radiation therapy already (Jordan and Hundley, 2019). LVEF function can also be measured when CMR is carried out on an individual, which can save a lot of time which can often be crucial in cancer patients (Jordan and Hundley, 2019). The main strengths of CMR

are that it has high spatial resolution and tissue analysis features. However, the main limitations of using this method is that it is an expensive machine and patients who have claustrophobia and/or cardiac implanted devices are unable to have CMR imaging (Awadalla *et al.,* 2018). Therefore, this method is not very accessible to patients due to the machines high cost and high knowledge user nature.

Cardiac Computed Tomography (CT)

Cardiac computed tomography is useful in the evaluation of masses, pericardial and coronary artery disease, however limitations include radiation exposure and limited knowledge and information on cardiotoxicity measures (Awadalla *et al.,* 2018). CT scans have a key role in being able to differentiate malignant tumours from benign tumours as well as being able to detect cardiac metastases (Seraphim *et al.,* 2019). Cardiovascular CT scans are used to assess cardiac chamber sizes, mass, left ventricle and right ventricle ejection fraction with good reproducibility (Podlesnikar *et al.,* 2022). The method also plays an important role at identifying any calcifications in the aortic, valvular, and myocardial areas (Podlesnikar *et al.,* 2022). The strengths of this method is that it is widely available in many hospitals and has a short scanning time (Kalisz and Rajiah, 2017). CT scans also have a high temporal and high spatial resolution which allows for the analysis of small structures such as the coronary arteries. However, the limitations of using this method is that it includes the use of ionising radiation and an iodinated contrast substance (Kalisz and Rajiah, 2017). Therefore, this is not the most suitable and safest method for cancer patients who already may be receiving radiation therapy, especially to the chest area as this may risk making the cardiotoxicity that the patient may have even worse.

Electrocardiogram (ECG)

ECG is used to detect and analyse the electrical activity of the heart (Liu *et al.,* 2021). The 12 lead ECG is a simple quick easy method that can provide information on cardiotoxicity, which mainly detects ischemic changes or arrhythmias. The most common side effects of chemotherapy that can be detected via the 12 lead ECG is sinus bradycardia (Spînu *et al.,* 2021). Sinus bradycardia can cause heart failure, chest pain, dizziness, confusion, fatigue and episodes of fainting if the heart is not effectively pumping blood around the body. Cardiotoxicity weakens the pumping function of the heart which in turn causes sinus bradycardia (Tamargo *et al.,* 2022). The strengths of the ECG is that it is easily accessible, quick, portable and simple to carry out. The limitations are that artefacts can occur, and interpretation of the results can be misread or misinterpreted (Schläpfer and Wellens, 2017). Whilst this method is useful for understanding the hearts conduction system, it cannot measure blood flow or always be accurately interpreted by medical professionals (Rafie *et al.,* 2021). The computerised interpretation of the ECG often provides incorrect readings for arrythmias, conduction disorders and pacemaker rhythms. This leads to a concern for false-positive results and false-negative results in the identification of ST-segment elevation myocardial infarction (STEMI) (Schläpfer and Wellens, 2017). In addition, there is a risk of over-reading on the ECG machine, so therefore continuous education is needed for members of staff (Schläpfer and Wellens, 2017). This may limit ECG use in cardiotoxicity detection.

Stress Myocardial Perfusion Imaging

Stress myocardial perfusion imaging can identify significant coronary artery disease and help understand risk before surgery or prior to having cancer treatment (Seraphim *et al.,* 2019). Stress myocardial perfusion imaging can involve a treadmill or involve a specific drug such as dobutamine, dipyridamole or adenosine that makes the heart perform as if you were exercising (Makaryus and Diamond, 2012). When assessing cancer patients and cancer survivors for cardiotoxicity, the heart muscle is analysed to see how it is coping under stress

and to see if there are any differences compared to healthy individuals (Seraphim *et al.,* 2019; Makaryus and Diamond, 2012). The limitation of this method is that beam hardening and motion artefacts may affect the accuracy of the scan, which may lead to errors in diagnosis or outcome (Seitun *et al.,* 2016). Beam hardening occurs when there are false lines on the image, indicating a non-existent density (Ramakrishna *et al.,* 2006). The strength of this method is that it has high spatial resolution, so this allows the detection of smaller defects and that the scan is only needed to be done once for all information to be gathered (Seitun *et al.,* 2016).

See table 2 for other methods that are used to measure and detect cardiotoxicity.

Table 2: Displays other methods that are used to measure and detect cardiotoxicity.

Method	Method Information	Strengths	Limitations
Endomyocardial Biopsy	• An endomyocardial biopsy (EMB). • Small part of heart muscle is removed and analysed to detect any tissue damage.	• Can help detect cardiac disorders: cardiomyopathies, amyloidosis, myocarditis, cardiac tumours, and drug related cardiotoxicity (Porcari *et al.,* 2022).	• Declining in use due to its invasive and costly nature (Huang *et al.,* 2022). • Used when findings on non-invasive procedures (MRI) are unclear (Huang *et al.,* 2022). • Invasive procedure.
Radionuclide Ventriculography (RVG)	• An accepted measurement of ejection fraction (Ro *et al.,* 2023; Jones *et al.,* 2020). • Used in patients who are undergoing cancer treatment to assess their left ventricle function	• Measures the heart non-invasively. • It can allow for assessment of the function of the heart before, during and after chemotherapy,	• Uses radiation to scan however it is minimal (Odak and Kayani, 2022). • Inaccessible method. • For breast cancer patients already undergoing radiation treatment this would be an extra dose to the chest.

	(Ro *et al.,* 2023; Jones *et al.,* 2020). • Various images of the heart are taken at various times throughout its contraction and relaxation stages (Odak and Kayani, 2022).	so this can allow for detection of cardiotoxicity.	• Can worsen the already reduced function of the heart, so can contribute to cardiotoxicity.
<u>Automatic Border Detection (ABD)</u>	• Used to evaluate volume measurements and left ventricular function in the heart (Hashimoto *et al.,* 1999). • Detects the endocardial border in the left ventricular cavity to then calculate left ventricular volumes and heart function (Jurcut *et al.,* 2008).	• None	• Frequent need to change the automatic algorithm which is involved in the image processing. • Method not as easily repeatable and routine compared to other methods available such as echocardiography (Jurcut *et al.,* 2008).

Echocardiographic Integrated Backscatter (IB)	• Ultrasound beams are scattered through the myocardium, then some of the scattered signals reflect back to the ultrasound probe (Tuohinen *et al.,* 2017). • cIBS measures the texture properties of the myocardium and then CVIBS measures the intrinsic contractility of the myocardium (Tuohinen *et al.,* 2017).	• Nagai and colleagues (2003) research posit that IB may be a useful tool for the detection of early cardiotoxicity.	• None
Positron Emission Tomography (PET)	• Useful to identify any metastatic lesion and assessments of the response to chemotherapy.	• Can identify cardiac dysfunction associated with heart failure (D'Amore et al., 2014).	• The use of PET scans in a clinical environment is limited due to the high user knowledge required, price

			of scanner and low availability (Simoni and Brandão, 2017). • PET scans are still debated in detecting cardiotoxicity, especially in the early stages (D'Amore *et al.,* 2014).
Myocardial Deformation (strain) Imaging	• Method that can show early contractile dysfunction in cardiovascular diseases (Scatteia *et al.,* 2017). • Method that looks at the total ventricular myocardial deformation in a whole cardiac cycle (Brady *et al.,* 2022).	• Better sensitivity than LVEF. • Can also diagnose myocardial ischaemia when used in conjunction with other methods (Smiseth *et al.,* 2015).	• Blood pressure needs to be considered when strain imaging measurements are being interpreted, due to the systolic strain being load dependent (Smiseth *et al.,* 2015). • The limitations: very much angle dependent because radial strain is polar opposite of the longitudinal and circumferential strains, this can

	• The method uses ultrasound wave frequency to measure strain rate, (how much the myocardial fibres stretch) (Brady *et al.*, 2022).		then cause underestimates in the absolute strain. • Lot of subjectivity in the interpretation of strain image results. • Usually used as a supplementary method on top of a more popular commonly used method (Smiseth *et al.,* 2015).
Dobutamine Stress Echocardiography Test (DSE)	• Low doses of dobutamine are injected into the blood stream via a cannula in the vein to investigate the contractile reserve of the myocardium (Jurcut *et al.,* 2008).	• Known to be reliable and non-invasive to assess left ventricular function (Cottin *et al.,* 2000).	• Test is controversial due to a range of different findings across researchers. • Civelli and colleagues (2006) found that the DSE test can identify cardiotoxicity by assessing the

	• Used more commonly and well established for the use in myocardial infarction patients.		contractile reserve of the myocardium. • Bountioukos and colleagues (2003) findings suggested that there is no value using the DSE test for detection of cardiotoxicity using contractile reserve. • Findings suggesting that DSE did not predict the systolic LV function after chemotherapy was finished. • This test is not commonly used in the oncology sectors (Jurcut *et al.,* 2008). • The test is still controversial for chemotherapy patients.

Doppler Myocardial Imaging (DMI)	• Sensitive method which allows measurement of myocardial velocity, strain rates/strain (Jurcut *et al.,* 2008). • Parameters strain and strain rate are considered the most valuable in the early detection of left ventricular dysfunction induced by chemotherapy drugs.	• Reliable to detect early abnormalities in global and regional myocardial function (Jurcut *et al.,* 2008). • In most modern cardiac scanners and extra test would only add a few more minutes onto the usual echo study test (Jurcut *et al.,* 2008).	• More research is needed to confirm cut off values and normal range measurements (Jurcut *et al.,* 2008).

2.9. Ultrasound

Ultrasound is a safe, affordable, and widely used diagnostic machine to analyse and evaluate images of the body produced through ultrasound waves (Phenix *et al.,* 2014). Brightness-mode imaging (B-mode) is used when scanning the body with ultrasound. This involves the transmission of ultrasound echo into the body part being scanned by the transducer. As the ultrasound echo waves penetrate the body's tissues some are reflected back to the transducer, and some continue to penetrate further into different tissues. The ultrasound echo waves that return to the transducer at different times are all combined to then create an image of the body part that is being scanned. The ultrasound machine is thought to work as a speaker due to it creating ultrasound echo waves and as a microphone due to it receiving the ultrasound echo waves (Phenix *et al.,* 2014).

Ultrasound is commonly used to monitor features of the carotid artery. These features include peak-systolic velocity (PSV) and end-diastolic velocity (EDV), intima-media wall thickness (IMT), and the diameter of the common carotid artery in transverse and longitudinal view. PSV measures blood flow velocity at its highest during systole, which can help understand how the heart is functioning when it is contracting. EDV measures blood flow velocity at its slowest during diastole, which can then help understand how the heart is functioning when it is relaxing. Any abnormal results of PSV and EDV can indicate that cardiovascular disease is present (Chuang *et al.,* 2015; Mari *et al.,* 2005). IMT it can help investigate if the heart is under any stress, as higher IMT values can indicate a diseased heart (Simova, 2015). The diameter of the CCA in transverse view and longitudinal view provides valuable information on the function of the CCA, as an enlarged CCA can be a predictor for future cardiac events and CVD (Jezovnik and Poredos, 2010).

Moreover, the ultrasound can be used to scan recovered breast cancer survivor's common carotid artery to assess blood flow velocity including peak-systolic velocity (PSV) and end-diastolic velocity (EDV), intima-media wall thickness (IMT), and the diameter of the common carotid artery in transverse and longitudinal view. This information may theoretically provide valuable insight into how the heart is functioning and how much blood the heart can pump at each contraction. Then in theory, this may allow for the detection and assessment of cardiotoxicity.

Chapter 3 - Methods

3.1. General Study Design

A quantitative, cross-sectional pilot measure study design was used to address the aims and objectives of this study (Capili, 2021). Three groups of participants were recruited: young healthy females, older non-malignant females, and female breast cancer survivors. All participants completed a health questionnaire (see appendix 1) which was used to identify if participants were fit to take part in the study and any participants which may be at risk taking part in the study. Once the individuals were identified and it was determined that it was safe for them to take part in the study, they then attended the lab once for a total of 30 minutes for all the measurements to be taken. Measurements such as mass (kg), height (cm), blood pressure (bp), heart rate (bpm) and date of birth were taken and recorded onto a secure document password protected database. After which, the primary measures for the study, including the participant's blood flow in their carotid artery were measured at rest using the GE Logiq e R8 ultrasound machine. All results were collected and safely stored afterwards onto a password protected database. Data was analysed via SPSS 28 and comparison were made between the groups.

3.2. Feasibility Assessment of the Study

Feasibility was assessed for this study by ensuring that there were sufficient numbers of participants available to recruit, this was done by researching breast cancer support groups beforehand and local community groups such as hiking groups for the older healthy females, and young healthy females in the university community. Therefore, it was concluded that the study was viable due to the ability to successfully recruit breast cancer survivors from the

community. A literature review and extensive research on this study was conducted beforehand to ensure that this study should allow to be successful. Ensuring that my supervisors expertise matched with the study was also a key factor in the ability to complete this study to a high standard. The imaging suite where the study took place was assessed for suitability for carrying out the study successfully. Suitability was ensured by making sure that the equipment was up to date and was sensitive enough to produce high quality images in all groups of the participants. The equipment was also ensured to be able to successfully detect differences of the common carotid artery between the groups. Laboratory support also had to be ensured to carry out the study, i.e. in case of emergencies as well as the accessibility of the imaging suite. Training qualifications of carotid ultrasound carried out by the researcher ensured that the scans were carried out correctly and to a sufficient standard so that data that was collected could be analysed and provide accurate conclusions.

3.3. Ethical Considerations

Durham University's ethics policy and ethical guidelines were strictly followed when carrying out this investigation. An ethics application was filled out and approved before undertaking any testing with participants. All participants were recruited on a volunteer basis, and consent documents were signed prior to any testing. The anonymity of participants was ensured by assigning each participant a number upon their consent to participate. Therefore, when communicating the data in the master's work, group data is presented or a randomly assigned number where appropriate. Confidentiality was ensured by not storing any identifying data with the participant data and storing all data that is collected on password protected documents. All links to the identifying data that was collected will be destroyed 6 months after the master's degree has been awarded. All risk assessments and procedures were

filled out and followed when completing the testing process. The tests conducted within the project were non-diagnostics in nature. If any unusual or concerning scans results were noted during the testing, the participant was informed, and a referral form (see appendix 3) was filled out to convey the study results to a medical professional with the lead supervisor's contact information and signature so that the participant could follow-up with a professional opinion and diagnosis from the GP.

It is also important to note that the breast cancer survivors have been recruited from the community and not via the NHS via a digital poster. This means that it was not necessary to seek HRA and IRAS ethical approval. The younger and older healthy females were recruited via the community, via a digital poster and word of mouth.

3.4. Participants (human)

Participants

Participants were recruited via criterion-based purposive sampling (Palinkas *et al.,* 2013). Participants that met the certain criteria and characteristics have been recruited through the community via a digital poster that was sent to charities, organisations, and community groups that help breast cancer survivors and the young healthy females were recruited via word of mouth to the undergraduate community at Durham University. The older healthy females were recruited via word of mouth and digital posters which were sent to relevant community groups.

Inclusion and Exclusion Criteria

Breast cancer survivors who had completed their primary treatment 1 year or more from the time of the study were eligible to take part. This is due to fact that cardiotoxicity typically

develops when a patient reaches 1 year or more after treatment, the cardiotoxicity and cardiac problems are often irreversible at this stage and can cause longer term problems with their heart (Bulten *et al.,* 2015; Jarcut *et al.,* 2008). In a breast cancer survivor that has had treatment within the past 11 months cardiac damage that may be present is more likely to be reversible and more short-term damage (Bulten *et al.,* 2015; Jarcut *et al*., 2008). This timeline also facilitated recruitment from community-based settings. Female breast cancer survivors had either chemotherapy, radiotherapy or hormonal treatment alone or in combination and were of any age. Given the limited timeline for the project (1 year) and the study being a pilot study, it was not possible to age match participants taking part in the study. Therefore, for accessibility reasons, any age in the recovered breast cancer survivors was accepted in this study.

Exclusion criteria for the young healthy females and older healthy females included current pregnancy, any past or current cancer diagnosis, any cardiovascular disease or any pulmonary lung diseases. This was to ensure that the common carotid artery results could be compared accurately to defined groups. The young healthy females were to be in the age category range of 18 – 30 years of age, this is due to normative data classing 30 years of age and below as in the "young" category (Rasif *et al.,* 2017; Marsh *et al.,* 2013). Moreover, older healthy females being in the age category of 50 years and above, due to it being the age in which breast cancer is mostly likely to develop (McPherson *et al.,* 2000).

Key Measures

Demographic characteristics, including, the participants age (years), heart rate (bpm), blood pressure (bp), body mass index (BMI), height (cm) and weight (kg) were used to characterise the participants and describe who took part in the pilot study. The measure to be investigated

as markers or cardiotoxicity included the primary markers of blood flow in the carotid artery, including, CCA diameter in transverse and longitudinal view, intima-media wall thickness and rate of flow (PSV and EDV) (Uematsu *et al.,* 1983). Breast cancer survivor details such as date of diagnosis, type of breast cancer, stage of disease, type of treatment, date of being in remission, and the date of being classed as cancer-free by medical professionals and oncologists were also collected (Chan *et al.,* 2014).

3.5. Procedures

Pre-Test Procedure

Once the participants were successfully screened by completing a health questionnaire to confirm they were eligible to take part in the study, participants provided their informed written consent and then data collection commenced. The participants age (years), heart rate (bpm), blood pressure (bp), body mass index (BMI), height (cm) and weight (kg) were collected and inserted onto a secure password protected file digitally. Age was recorded onto a spreadsheet after the consent form was signed which was done by recording the participants date of birth, heart rate (bpm) and blood pressure (bp) were collected via an automated blood pressure machine (Omron M2 Basic - Omron Healthcare Europe B.V., Hoofddorp, Netherlands). Heart rate and blood pressure were collected by asking the participant to sit down quietly for 5 minutes and then the blood pressure cuff was put on the participant with their consent, and the start button was pressed. Participants were asked not to talk and relax when the blood pressure machine was taking the measurements. The heart rate and blood pressure were then recorded onto a secure spreadsheet. Heart rate and blood pressure were taken three times, and an average was then calculated. Height (cm) was collected using the stadiometer (Manufacturer – Seca, Model - 213) and weight (kg) was collected using standard weighing scales in (kg) (Manufacturer – Seca, Model – 875). Height was taken by

asking the participants to stand with the back of their head, shoulder blades, buttocks and heels touching the stadiometer. Participants were asked to stand as straight as possible, as well as having no up-do hair styles. The head plate was then brought down to rest on the crown of the head and the measurement was taken and recorded onto the secure spreadsheet. Weight was taken by firstly asking the participants to remove their shoes and jacket/coat. They were then asked to step onto the weighing scale and stand still in the centre of the weighing scale looking straight ahead and with a good posture. The reading was then inserted onto the secure spreadsheet. BMI was calculated manually through dividing their weight (kg) by their height (m) to then give a BMI value. Breast cancer survivor details were also collected with details such as date of diagnosis, type of breast cancer, stage of disease, type of treatment, date of being in remission, and the date of being classed as cancer-free by medical professionals and oncologists (Chan *et al.,* 2014). This information was collected via self-report through a questionnaire that was given to all breast cancer survivors prior to attending the data collection appointment (see appendix 2).

Carotid Artery Ultrasound Examination

The test was undertaken on the GE Logiq e R8 ultrasound machine and using the GE Linear Array L4-12t-RS Transducer, with an imaging frequency of 4.2-13.0 MHz. Measurements of the right common carotid artery (CCA) including the diameter of the CCA in transverse view, diameter of the CCA in longitudinal view, carotid intima-media thickness (IMT) was taken (Galderisi *et al.,* 2007). The peak systolic velocity (PS) and end diastolic velocity (ED) of the CCA were also then measured. The right common carotid artery arising from the right brachiocephalic artery was used for this study because carotid ultrasound can show the most proximal section of the common carotid artery (Lee, 2014). In contrast, carotid ultrasound of the left common carotid artery arising from the aortic arch cannot show the proximal section of the left common carotid artery (Lee, 2014).

3.6. Measurements Explained

Diameter of the CCA in Transverse View and Longitudinal View

The diameter of the CCA in transverse view is taken by using an ultrasound machine and it is completed by firstly locating the thyroid, and then measuring the top of the CCA wall to the bottom of the CCA wall with the measurement function on the ultrasound machine (see figure 4). This was repeated three times, and an average was then calculated. The diameter of the CCA in longitudinal view is taken again by locating the thyroid and finding the CCA in transverse view but then turning the ultrasound probe clockwise to get a tube-like image on the ultrasound screen. Then the top of the CCA intima media wall to the bottom of the CCA intima media wall distance is measured using the measurement function on the ultrasound machine (see figure 5). This again was repeated three times, and an average was then calculated. There is rising evidence to suggest that different stimuli can cause a change in the diameter of arteries, including the common carotid (Jezovnik and Poredos, 2010). Different stimuli include haemodynamic stress, injury, and inflammation. Therefore, the diameter of the CCA in transverse view and longitudinal view was included in the study due to cardiotoxicity causing all three of these stimuli. Moreover, the enlargement of carotid arteries can imply the risk of cardiovascular disease and events as during the atherosclerotic process the diameter of the artery increases (Jezovnik and Poredos, 2010). The normative measurements of the diameter of the CCA that would be expected is 6.10 mm ± 0.80 (Krejza *et al.,* 2006). Furthermore, carotid artery diameter can also be influenced by blood pressure and related to body height and weight independent of sex (Krejza *et al.,* 2006).

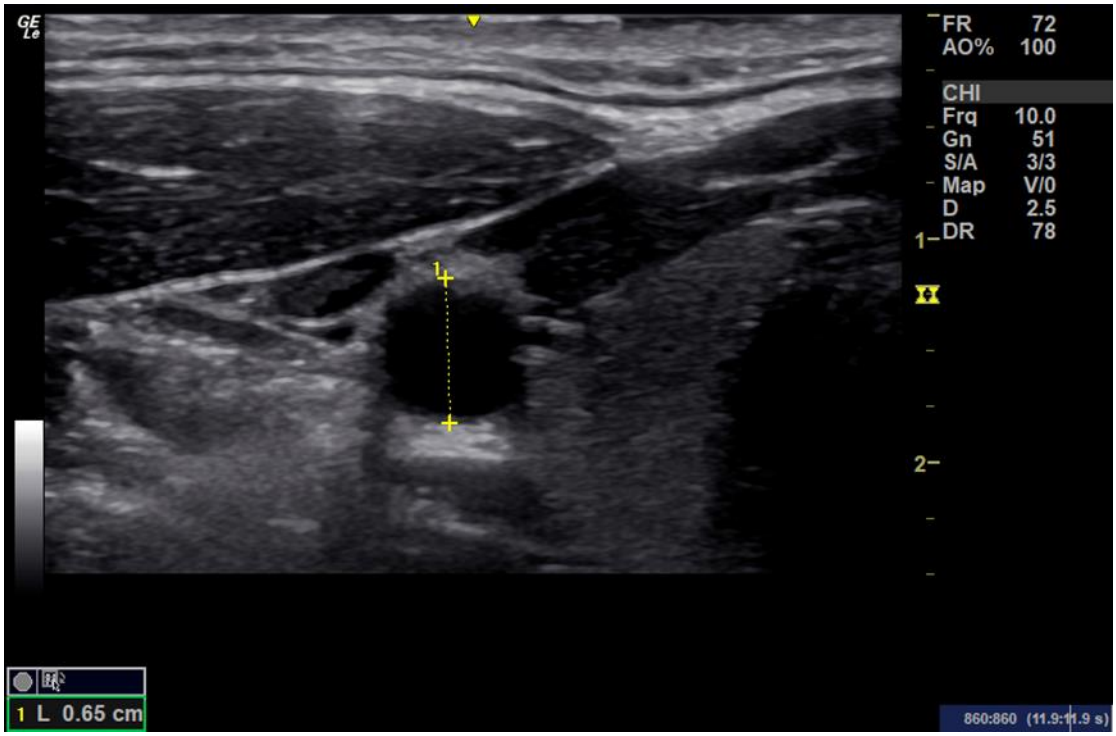

Figure 4. Displays the diameter of the CCA in Transverse View. The measure function has measured from the top of the CCA to the bottom of the CCA.

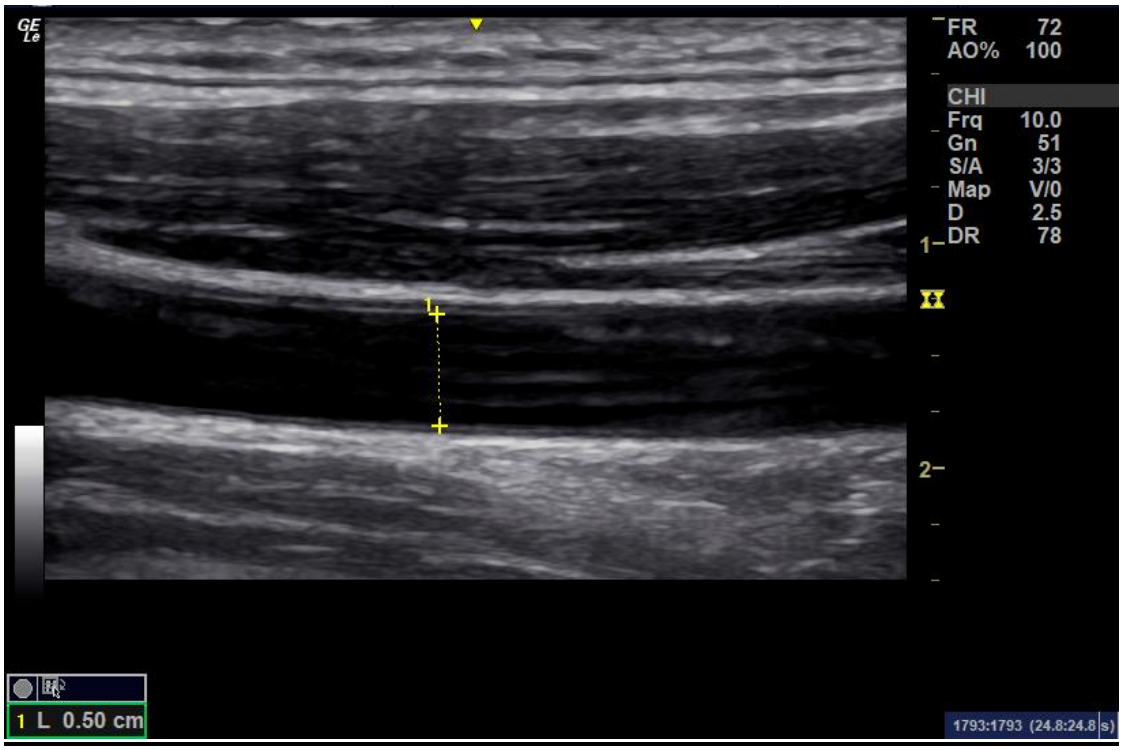

Figure 5. Displays the diameter of the CCA in Longitudinal View. The measure function has measured from the top lumen–intima (LI) interface to the bottom lumen–intima (LI) interface of the CCA.

Intima-Media Wall Thickness

Intima-media wall thickness was taken by firstly locating the thyroid with the ultrasound machine and then finding the CCA in transverse view. Then the ultrasound probe was turned clockwise to get the CCA in longitudinal view, and then the bottom intima media wall was measured using the measurement function on the ultrasound machine. The measurement was taken from the lumen–intima (LI) and the media–adventitia (MA) interfaces. The IMT measurements were taken proximal to the carotid bulb (Simova, 2015) (See figure 6). This was repeated three times, and an average was then calculated.

Common carotid artery intima-media thickness is known as a marker for atherosclerosis (van den Munckhof *et al.,* 2018). Absolute IMT value thresholds are however controversial in the literature and instead measurements are usually compared to a percentile ranges (Simova, 2015). Nevertheless, it is important to note that in the most recent ESC hypertension guidelines (2013), carotid IMT that is 0.9mm and above has been confirmed as a marker for asymptomatic damage to organs. In contrast, the American society of Echography states that IMT higher or equal to the 75^{th} percentile is considered high and is an indicator of a higher risk of cardiovascular disease (Simova, 2015) (See table 3). Therefore, in this study the standard of measurements has been considered when analysing the IMT data.

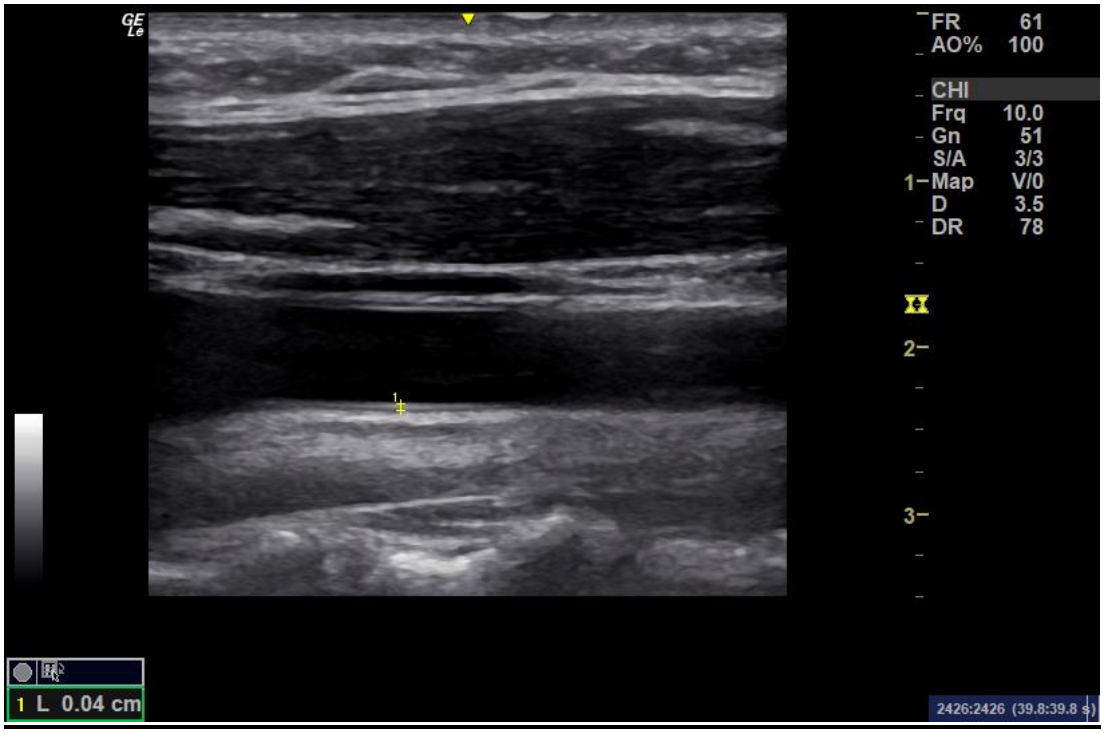

Figure 6. Displays the measurement of the carotid intima-media wall thickness. The measurement is from the lumen–intima (LI) and the media–adventitia (MA) interfaces.

Table 3: Right Common Carotid Artery IMT Values for Females (Table adapted from Simova, 2015).			
Females	*25th Percentile*	*50th Percentile*	*75th Percentile*
Aged 30 and below	0.39 mm	0.40 mm	0.43 mm
Aged 31-40	0.42 mm	0.45 mm	0.49 mm
Aged 41-50	0.44 mm	0.48 mm	0.53 mm
Aged 50 years and above	0.50 mm	0.54 mm	0.59 mm

PSV and EDV of the CCA

PSV and EDV were taken on the ultrasound machine by locating the thyroid and viewing the CCA in transverse view, and then turning the ultrasound probe clockwise to view the CCA in longitudinal view. Then the colour button was pressed and then the doppler button was pressed whilst still over the CCA in longitudinal view. The freeze button was then pressed so that the PSV and EDV results could be recorded onto a secure spreadsheet (see figure 7). This was repeated three times, and an average was then calculated. Peak systolic velocity is the blood flowing at its highest velocity during systole and end diastolic velocity is the blood flowing at its slowest velocity during diastole (Chuang *et al.,* 2015; Mari *et al.,* 2005). Peak systolic velocity and end diastolic velocity are valuable measures when looking at how blood flows through the carotid artery (Kim *et al.,* 2020). Abnormalities with both the CCA waveform and PSV and EDV measurements can indicate disease. A lower PSV value may indicate that the heart is not pumping enough blood during systole, similarly to EDV if a value was abnormally low during diastole. Cardiotoxicity can reduce the function of the heart, causing the heart to pump a smaller volume of blood during systole (Kim *et al.,* 2020).

Figure 7. Displays the PSV and EDV of the common carotid artery as well as the waveform of the CCA.

Moreover, waveforms that do not follow a regular structure and pattern may indicate arrythmia. Abnormal waveforms may also indicate cardiovascular problems, therefore helping to potentially detect any cardiotoxicity that may be present. See figure 8 below for representation of a normal waveform figure in the CCA.

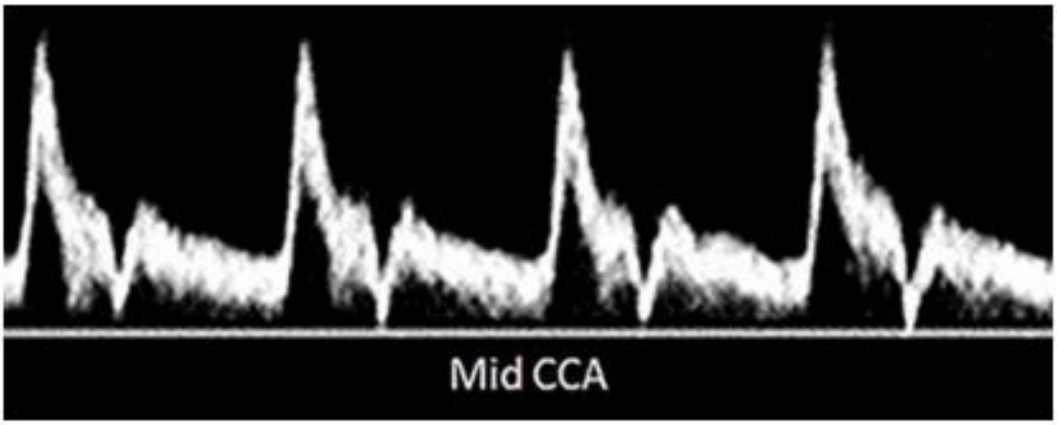

Figure 8. Displays the normal waveform figure in the CCA. Image from: (Kim *et al.,* 2020).

3.7. Analysis

All data was collected and a mean ± SD for each measure for each group was presented. Due to the cross-sectional nature of the study design the analysis included comparing differences between each group. First, a normality test was conducted with all the data and the measures that were parametric were analysed via a One-way ANOVA. The parametric data included height (cm), weight (kg), BMI (kg/cm^2), systolic, diastolic, diameter of CCA in longitudinal view (cm), PSV (cm/s) and EDV (cm/s). The measures that were non-parametric were analysed via a Kruskal-Wallis test. The non-parametric data included heart rate (bpm), diameter of CCA in transverse view (cm) and intima media wall thickness (IMT) (cm). The Levene's test was also used in the Kruskal-Wallis test to assess the homogeneity of variance. The post-hoc test that was used in the analysis was Tukey. Excel software was used to help clean and evaluate the data, while SPSS v. 28 analysed the data. Confidence intervals have been added onto each result to allow for an understanding of the reliability of the sample estimates. An ANCOVA was completed where BMI was controlled and the markers that were tested were PSV, EDV, IMT, CCA in transverse view and CCA in longitudinal view.

Chapter 4 - Results

4.1. Pilot Outcomes

The study was completed as planned. From the participants recruited, 34 participants were screened, 30 participants were eligible to participate in the study and were distributed equally (n=10), for each group. See below in table 4. After attempting to recruit participants for 1 month [4 weeks], the inclusion criteria were expanded to all treatment types which included chemotherapy, radiotherapy and hormone therapy, as it was not possible to find enough breast cancer survivors that had chemotherapy alone. There were two participants that had abnormal scan results in the data set, so those individuals were then promptly referred to their GP with an 'incidental findings and GP referral form'. The two individuals with abnormal scan results were then excluded from the study as they no longer met the older healthy female inclusion criteria.

Feasibility Outcomes of the Study

Participants, equipment used, accurate and reliable results supported that this study was feasible to conduct. There were enough participants available for the study for each group. Participants were successfully recruited via the community for this study, which shows it was feasible to conduct a sufficient number of participants to take part. It must be noted that the inclusion criteria had to be expanded for this study to be successful, where breast cancer survivors did not just need to only have had chemotherapy as treatment alone, but could have had chemotherapy, radiotherapy and/or hormonal therapy. The equipment used yielded quality measurements enabling accurate and reliable results to be gained. It successfully showed proof of concept, successful design and experiment feasibility and lastly successful capability and capacity analysis.

Table 4 – Mean Descriptive Characteristics

	Young Healthy Females (18-30 years)	*Older Healthy Females (50 years and over)*	*Breast Cancer Survivor Females (any age)*
Age (years)	21.6 ± 2.12 (CIS (LB) 20.01 – (UB) 23.19)	61.2 ± 6 (CIS (LB) 56.68 – (UB) 65.72)	59.8 ± 10.98 (CIS (LB) 51.52 – (UB) 68.08)
Height (cm)	164.89 ± 6.23 (CIS (LB) 160.43 – (UB) 169.35)	162.09 ± 5.17 (CIS (LB) 158.39 – (UB) 165.79))	160.45 ± 4.59 (CIS (LB) 157.17 – (UB) 163.73)
Weight (kg)	60.08 ± 10.37 * (CIS (LB) 52.66 – (UB) 67.50)	64.98 ± 8.46 ‡ (CIS (LB) 58.93 – (UB) 71.03)	76.76 ± 9.79 (CIS (LB) 69.76 – (UB) 83.76)
BMI (kg/cm^2)	22.09 ± 3.51* (CIS (LB) 19.58 – (UB) 24.60)	24.73 ± 2.99‡ (CIS (LB) 22.59 – (UB) 26.87)	29.82 ± 3.74 (CIS (LB) 27.14 – (UB) 32.50)
Heart Rate (bpm)	74 ± 16 (CIS (LB) 62.00 – (UB) 85.20)	71 ± 14 (CIS (LB) 61.19 – (UB) 81.21)	76 ± 11 (CIS (LB) 67.80 – (UB) 84.20)
Systolic Blood Pressure	107.5 ± 13.25*† (CIS (LB) 98.02 – (UB) 116.98)	132.3 ± 11.97 (CIS (LB) 123.74 – (UB) 140.86)	133.1 ± 13.08 (CIS (LB) 123.75 – (UB) 142.45)
Diastolic Blood Pressure	70 ± 8.72*† (CIS (LB) 63.76 – (UB) 76.24)	82.7 ± 6.63 (CIS (LB) 77.95 – (UB) 87.45)	83.4 ± 7.66 (CIS (LB) 77.92 – (UB) 88.88)

Table 4: Displays the mean descriptive characteristics of age (years), height (cm), weight (kg), BMI (kg/cm), heart rate (bpm), systolic blood pressure and diastolic blood pressure. * *Indicating difference between Young Healthy (YH) and Breast Cancer Survivors (BCS), † indicating a difference Young Healthy (YH) and Older Healthy (OH), and ‡ indicating difference between Older Healthy (OH) and Breast Cancer Survivors (BCS).*

***Table 5* - Summary Table of Clinical Characteristics of Breast Cancer Survivors**

Survivors Time since Breast Cancer Diagnosis	2 years to 21 years (2003 – 2022) **Mean**: 2017 **Standard Deviation:** 5.80 **Range:** 19
Range of Treatments Given	**Surgery:** 7 BCS **Chemotherapy:** 4 BCS **Radiotherapy:** 9 BCS **Hormonal Treatments:** 7 BCS
Time range of breast cancer survivors being classed as cancer free	1 year to 21 years (2003 – 2023) **Mean:** 2018 **Standard Deviation:** 7.69 **Range:** 20
Last Dose of Cancer Treatment	2 years to 20 years (2004 – 2022) **Mean:** 2017 **Standard Deviation:** 5.57 **Range:** 17
The range of grades that the breast cancer survivors were diagnosed with	**Grade 0:** 1 BCS **Grade 1:** 3 BCS **Grade 2:** 4 BCS **Grade 3:** 2 BCS

Table 5: Displays a summary table of the clinical characteristics of the breast cancer survivors who took part in this pilot study.

4.2. Diameter of the CCA in Longitudinal View

The younger healthy females (0.52 cm ± 0.03) (CIS lower bound (LB) 0.50 – upper bound (UB) 0.54) had the smallest diameter of the CCA in longitudinal view, closely followed by the older healthy females (0.53 cm ± 0.06) (CIS (LB) 0.49 – (UB) 0.57), and then again followed closely by the breast cancer survivors (0.55 cm ± 0.04) (CIS (LB) 0.52 – (UB) 0.57). The breast cancer survivors had the largest diameter of the CCA in longitudinal view compared to all the groups. However, these differences did not reach significance (P = 0.425) See figure 9 below.

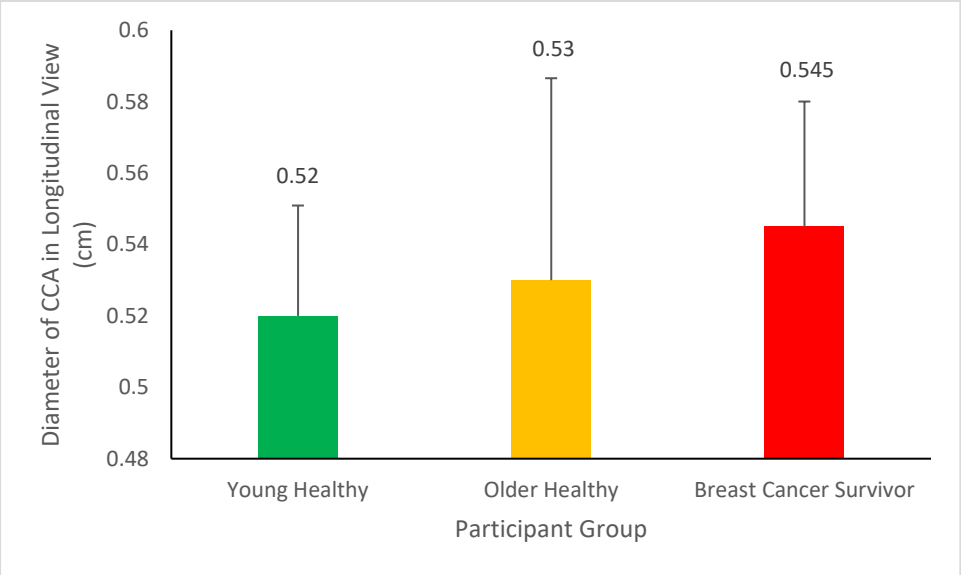

Figure 9. Diameter of CCA in Longitudinal View (cm) results of young healthy females, older healthy females, and female breast cancer survivors. * *Indicating difference between YH and BCS, † indicating a difference YH and OH, and ‡ indicating difference between OH and BCS.*

4.3. Diameter of the CCA in Transverse View

The younger healthy females (0.60 cm ± 0.04) (CIS (LB) 0.57 – (UB) 0.63) had the smallest diameter of the CCA in transverse view, closely followed by the older healthy females (0.63 cm ± 0.07) (CIS (LB) 0.58 – (UB) 0.68), and then again followed by the breast cancer survivors (0.65 cm ± 0.05) (CIS (LB) 0.62 – (UB) 0.69). The breast cancer survivors had the largest diameter of the CCA in transverse view compared to all the groups. However, these differences did not reach significance (P = 0.077). See figure 10 below.

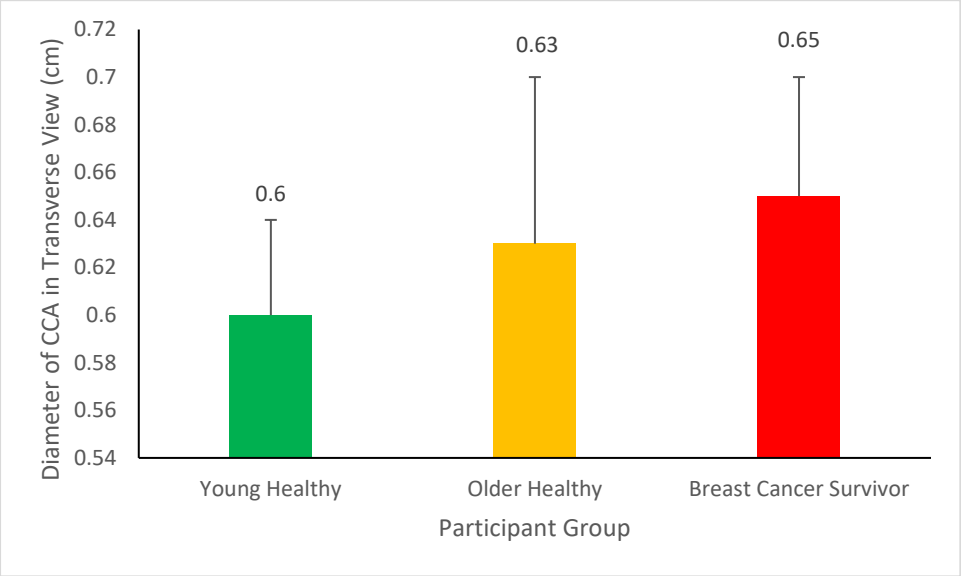

Figure 10. Diameter of CCA in Transverse View (cm) results of young healthy females, older healthy females, and female breast cancer survivors. * *Indicating difference between YH and BCS, † indicating a difference YH and OH, and ‡ indicating difference between OH and BCS.*

4.4. Intima Media Wall Thickness of the CCA

The younger healthy females (0.03 cm ± 0.01) (CIS (LB) 0.03 – (UB) 0.04) had the lowest intima media wall thickness value followed by the older healthy females (0.05 cm ± 0.01) (CIS (LB) 0.05 – (UB) 0.06) and breast cancer survivors (0.05 cm ± 0.01) (CIS (LB) 0.05 – (UB) 0.06). The older healthy females and breast cancer survivors had the same intima media

wall thickness value of 0.05 cm. There was significance between the young healthy females and older healthy females (P = < 0.001) as well as the young healthy females and breast cancer survivors (P = < 0.001). However, there was no significance between the older healthy females and breast cancer survivors (P = 0.644). See figure 11 below.

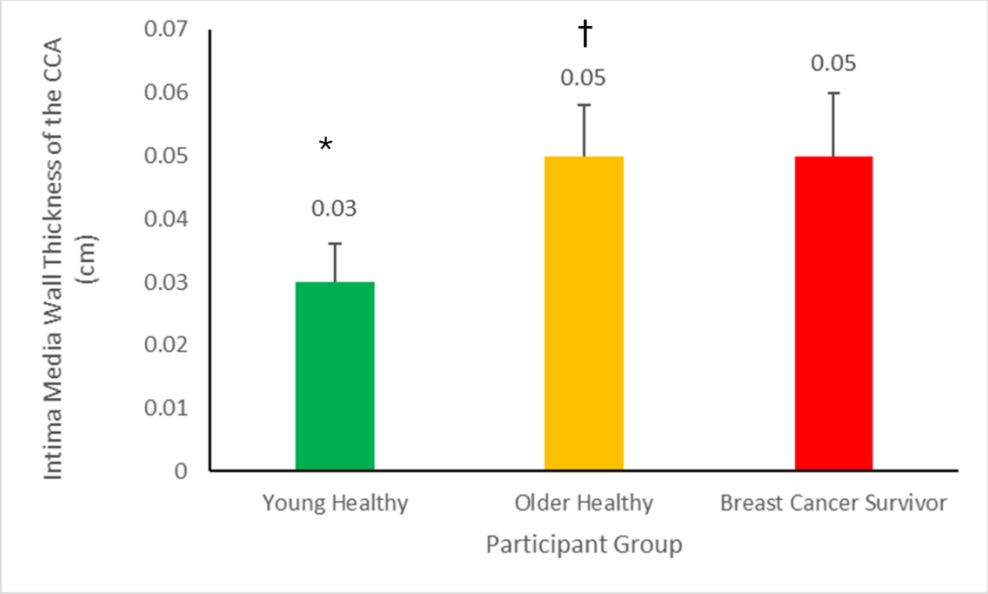

Figure 11. * † Intima Media Wall Thickness of the CCA (cm) results of young healthy females, older healthy females, and female breast cancer survivors. * *Indicating difference between YH and BCS, † indicating a difference YH and OH, and ‡ indicating difference between OH and BCS.*

4.5. Peak Systolic Velocity in the CCA

The younger healthy females (95.70 cm/s ± 14.42) (CIS (LB) 85.38 – (UB) 106.01) had the highest peak systolic velocity value followed by the older healthy females (80.95 cm/s ± 16.51) (CIS (LB) 69.13 – (UB) 92.76), and then again followed by the breast cancer survivors (73.61 cm/s ± 12.22) (CIS (LB) 64.86 – (UB) 82.35). The young healthy females (95.70 cm/s ± 14.42) (CIS (LB) 85.38 – (UB) 106.01) had the highest peak systolic velocity

value compared to all the groups. However, these differences did not reach significance (P = 0.077). See figure 12 below.

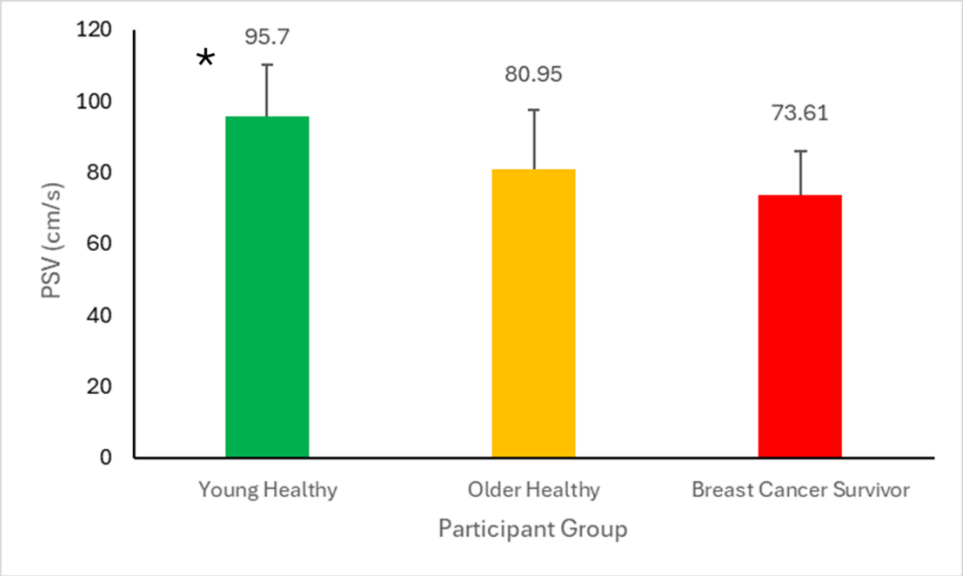

Figure 12. * PSV (cm/s) results of young healthy females, older healthy females, and female breast cancer survivors. * *Indicating difference between YH and BCS, † indicating a difference YH and OH, and ‡ indicating difference between OH and BCS.*

4.6. End Diastolic Velocity in the CCA

The older healthy females (22.72 cm/s ± 4.31) (CIS (LB) 19.63 – (UB) 25.81) had the highest end diastolic velocity value followed by the young healthy females (21.81 cm/s ± 5.76) (CIS (LB) 17.68 – (UB) 25.93), and then again followed by the breast cancer survivors (18.95 cm/s ± 4.94) (CIS (LB) 15.41 – (UB) 22.48). The older healthy females (22.72 cm/s ± 4.31) (CIS (LB) 19.63 – (UB) 25.81) had the highest end diastolic velocity value compared to all the groups, however this essentially equivalent to the younger healthy females (21.81 cm/s ± 5.76) (CIS (LB) 17.68 – (UB) 25.93). However, the differences between these groups and the breast cancer survivors did not reach significance (P = 0.236). See figure 13 below.

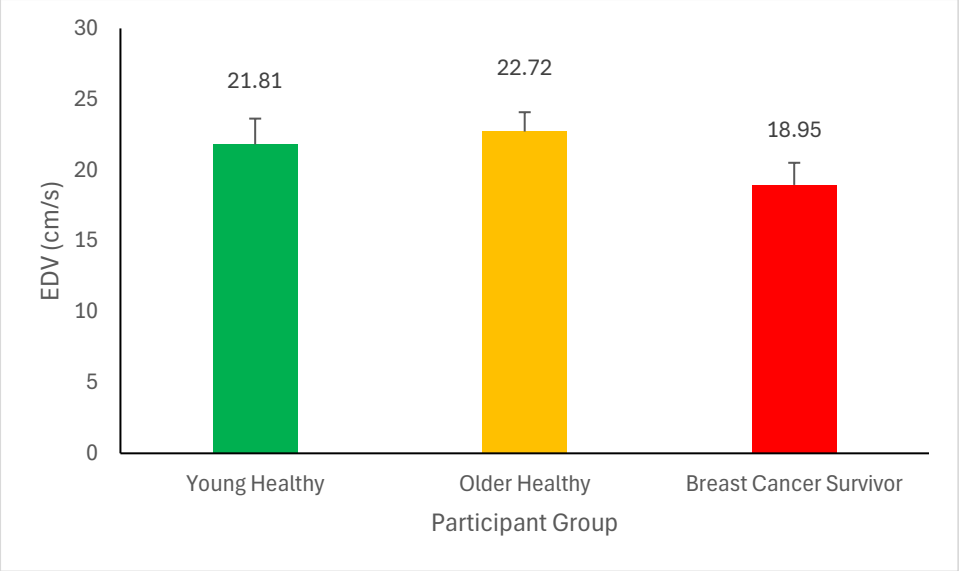

Figure 13. EDV (cm/s) results of young healthy females, older healthy females, and female breast cancer survivors. * *Indicating difference between YH and BCS, † indicating a difference YH and OH, and ‡ indicating difference between OH and BCS.*

ANCOVA Results: Controlling for BMI, as a 'supplementary' analysis

CCA in Longitudinal View

When BMI is controlled for the measure of the common carotid artery in longitudinal view it is of no significance and shows no difference between the groups, $F = 0.132$, $p = 0.877$, partial $\eta^2 = 0.010$.

CCA in Transverse View

When BMI is controlled for the measure of the common carotid artery in transverse view it is of no significance and shows no difference between the groups, $F = 1.214$, $p = 0.313$, partial $\eta^2 = 0.085$.

EDV of CCA

When BMI is controlled for the measure of end diastolic velocity in the common carotid artery it is of no significance and shows no difference between the groups, $F = 0.698$, $p = 0.506$, partial $\eta^2 = 0.051$.

PSV of CCA

When BMI is controlled for the measure of peak systolic velocity in the common carotid artery it is of no significance and shows no difference between the groups, $F = 2.137$, $p = 0.138$, partial $\eta^2 = 0.141$.

IMT of the CCA

When BMI is controlled for the measure of intima media wall thickness in the common carotid artery it is of significance and shows a difference between groups, $F = 11.135$, $p = < 0.001$, partial $\eta^2 = 0.461$.

Chapter 5 – Discussion

5.1. Discussion

The aim of this project was to investigate heart health by comparing differences in carotid artery blood flow parameters between breast cancer survivors and controls. The ultrasound measurements that were taken included PSV, EDV, diameter of CCA in longitudinal view and transverse view. Key findings from this work suggest that ultrasound may be a viable method to detect cardiotoxicity in breast cancer survivors. PSV was found to be significantly higher in younger healthy females when compared to breast cancer survivors. EDV was not found to be of significance between any groups. Resting heart rate average, systolic and diastolic blood pressure also appeared to be highest in breast cancer survivors, though these differences did not reach a statistical significance. Together these patterns may suggest that the heart of breast cancer survivors may be less efficient and is working harder, in this case more beats per minute and under more pressure, to pump blood around the body. This may be linked to the increased values in intima media wall thickness in the breast cancer survivors compared to young healthy participant. However, although there was a difference between the breast cancer survivors and the young healthy females, it is important to note that there was no difference between the older healthy females and breast cancer survivors. It is not possible to distinguish differences in outcomes due to cancer and the general aging process. This may be due to a small sample size that was used in this pilot study. It was not feasible to recruit a larger sample size in the one year in duration of the research master's degree.

Peak Systolic Velocity

Declines in peak systolic velocity of the CCA can show abnormalities of the heart such as cardiac arrhythmias, aortic stenosis and congestive heart failure (Bendick, 2014). PSV average was highest in the young healthy females (95.70 cm/s ± 14.42), followed by the older

healthy (80.95 cm/s ± 16.51) with and then lastly with the lowest PSV it was the breast cancer survivor group (73.61 cm/s ± 12.22). There was statistical significance between the young healthy females and breast cancer survivors, however there was no statistical significance between older healthy females and breast cancer survivors or between young healthy and older healthy females. Younger healthy females had a higher PSV than the older healthy females (16.7 % difference) and the older healthy females had a higher PSV than breast cancer survivors (9.5 % difference). However, the results demonstrate a clear pattern in PSV, with breast cancer survivors demonstrating the lowest PSV.

Normative PSV values are around 96 ± 25 (Trihan *et al.,* 2020). A lower PSV value indicates that there is a low or decreased cardiac output, meaning that the heart cannot pump an adequate volume of blood to meet the body's metabolic demands (Silva *et al.,* 2023; Oglat *et al.,* 2018). A low PSV value in the CCA has been associated with future cardiovascular disease (Chuang et al., 2015). While all of the groups are within this range, the breast cancer survivors are at the lowest end.

No one has measured PSV in breast cancer survivors before, but this result echoes the general idea of findings from König and Colleagues (2021) who examined PSV in individuals with and without coronary heart disease. König and Colleagues (2021) found that there was a difference between mean peak systolic velocity in the CCA between individuals with coronary heart disease and individuals without coronary heart disease. The results showed that individuals with coronary heart disease had a lower peak systolic velocity than those without coronary heart disease. However, it must be noted that even though the participants were not breast cancer survivors, it does support the findings that a heart in a diseased state does affect PSV in the CCA. As both cardiotoxicity and coronary heart disease both have direct effects on blood flow in the cardiovascular system this is unsurprising (Babiker *et al.,* 2018; Libby and Theroux, 2005).

With all of this considered, when BMI is controlled for the measure of peak systolic velocity in the common carotid artery shows no difference between the groups. This shows that the significant differences disappear when you control for BMI. Future work should BMI match breast cancer survivors and older females to see if there are differences. It must be noted that treatment is associated with weight gain (De Cicco *et al.,* 2019), which may contribute to the differences in BMI observed in the current study and this may also be confounding. This is necessary to determine if there are cancer specific differences that persist in a larger more controlled population. It is also important to note the heterogeneity of the breast cancer survivor population, such as different times of diagnosis, treatment variety and age etc., therefore future investigations need to be highly controlled. This may contribute to the variation observed in the current work.

End Diastolic Velocity

End diastolic velocity of the CCA is a helpful measure when understanding the health of the cardiovascular system, as it can detect conditions such as internal carotid artery (ICA) stenosis (Kamouchi *et al.,* 2005). EDV average was the highest in the older healthy group (22.72 cm/s ± 4.31), followed by the young healthy group (21.81 cm/s ± 5.76), and finally the lowest being breast cancer survivors (18.95 cm/s ± 4.94). No statistical significance was identified. Normative EDV values are 26 ± 6 (Trihan *et al.,* 2020; Oglat *et al.,* 2018). This indicates that breast cancer survivors on average had a lower-than-normal EDV value. An EDV value of 19 cm/s or below in the CCA is associated with a 64 % probability of a 70 % to 99 % internal carotid artery stenosis (Strosberg *et al.,* 2017). A low EDV value in the CCA has been associated with future cardiovascular disease (Chuang et al., 2015). The low value of EDV in the breast cancer survivors may suggest that the cancer treatment has had a

negative effect on their heart health and suggest that cardiotoxicity may be present in some of the breast cancer survivors. It also supports the research that suggests that breast cancer survivors have a higher risk of developing cardiovascular disease (Cherukuri *et al.,* 2022).

Intima-Media Wall Thickness

Intima media wall thickness of the CCA has been shown to predict future cardiovascular events, as the thicker the intima media wall, the higher the risk of a myocardial infarction or stroke (Coll and Feinstein, 2008). IMT is also an accepted marker of atherosclerosis (Coll and Feinstein, 2008). The intima media wall thickness average was not significantly different between breast cancer survivors and the older healthy group (0.05 cm ± 0.01), while the young healthy groups average measure was (0.03 cm ± 0.01). There was a statistical significance between breast cancer survivors and young healthy females as well as the older healthy females and younger healthy females. However, there was no statistical significance between breast cancer survivors and older healthy females.

These differences are likely due to age related factors. IMT normative values are classified in percentiles, with a value between the 25^{th} and 50^{th} percentile considered normal. In young (<30 years old) IMT normative values are : 1) 0.039 cm – 0.04 cm , and between 2) 0.05 cm – 0.054 cm for females >50 years old (Simova, 2015). The intima media wall thickness is classed as normal for older healthy females in the current study. However, due to the breast cancer survivors being any age, this has meant that we cannot detect a clear age category to compare the average to. Breast cancer survivors ranged from (41 – 77 years of age). In one of the younger breast cancer survivors (aged: 48) the intima media wall thickness was classed as high (0.06 cm). Normative values should be between 0.04 cm – 0.048 cm for females aged between 41 – 50 years old (Simova, 2015). This may suggest that there has been an effect from cancer treatment on the IMT wall thickness in the youngest breast cancer survivor. This

is concerning as higher IMT is directly linked to a higher risk for major cardiovascular events such as a myocardial infarction. This may mean that there is a higher risk for younger breast cancer survivors of experiencing a major cardiovascular event compared to women of the same age who have not had breast cancer. This may suggest that there is some process of premature aging of the heart from cancer treatments, however, much more research is needed to investigate this relationship further, as this speculation is based off a single participant. There is also research to suggest that intima media wall thickness and blood pressure may be linked (Vicenzini *et al.,* 2007). Future research with age matching participants must be considered to identify a clear difference between age-related changes in IMT or potential cardiotoxicity-related causes. As well as investigating the differences between older and younger breast cancer survivors, will also be needed to identify if cancer treatment influences premature aging of the heart. When BMI is controlled for the measure of intima media wall thickness in the common carotid artery it is of significance and shows a difference between groups. This again shows that the significant differences still exist when you control for BMI.

Diameter of the CCA in Longitudinal View and Diameter of the CCA in Transverse View

Having a larger CCA diameter is associated with a higher risk of cardiovascular disease and mortality (Sedaghat *et al.,* 2018). This larger CCA is thought to occur in individuals that have had their vascular wall affected by pulsatile blood pressure (for example thickening of the media), which is thought to occur to compensate for vascular wall stress (Sedaghat *et al.,* 2018).

Diameter of CCA in longitudinal view was the largest in breast cancer survivors (0.55 cm ± 0.04) closely followed by the older healthy females (0.53 cm ± 0.06) and young healthy females with 0.52 cm ± 0.03. Normative values for the diameter of the CCA in longitudinal

view is 0.54 cm ± 0.07 (Scuteri *et al.,* 2012). All groups were classed as having a normal diameter of the CCA in longitudinal view. There was no statistical significance found between the groups. This finding was unexpected due to previous research conducted by Eigenbrodt and colleagues (2006) stating that age is positively associated with common carotid artery diameter. Because of this, it was expected that the older healthy females and breast cancer survivors would have a larger CCA diameter due to the ageing process and potential premature aging process, that cancer treatment may cause to the heart. Though it is not of statistical significance it must be noted that breast cancer survivors do have the highest CCA diameter value when compared to all of the groups, with older healthy and young healthy females closely following.

Similarly, the diameter of CCA in transverse view average was highest in the breast cancer survivors (0.65 cm ± 0.05), again closely followed by the older healthy females (0.63 cm ± 0.07) and then young healthy females (0.60 cm ± 0.04). There are no normative values for the diameter of the CCA in transverse view. There was no statistical significance between the groups, this again was not expected for the same reasons discussed above as the diameter of the CCA in longitudinal view. Overall, in female breast cancer survivors, a larger carotid artery diameter may indicate cardiotoxicity. It has also been suggested that CCA diameter is influenced by blood pressure (Krejza *et al.,* 2006). Which also contributes to the unexpected findings as the breast cancer survivors had a high blood pressure on average.

Diastolic Blood Pressure and Systolic Blood Pressure

Diastolic blood pressure is when the heart is relaxed between beats, and this measurement represents the lowest pressure within the arteries (Shahoud *et al.,* 2023). Normal diastolic values should be 80 or below (Lüscher, 2018). Diastolic blood pressure results were classed as pre-hypertension in older healthy females (82.7± 6.63) and breast cancer survivors (83.4 ±

7.66). Young healthy females had a normal average diastolic blood pressure value. There was no statistical significance between older healthy females and breast cancer survivors, however there was statistical significance between the young healthy females and older healthy females, as well as the young healthy females and breast cancer survivors. This again, may be due to age related factors as mentioned previously.

Similarly, systolic blood pressure results were again classed as pre-hypertension in older healthy females (132.3 ± 11.97) and again in breast cancer survivors (133.1 ± 13.08). Young healthy females systolic blood pressure was classed as normal (107.5 ± 13.25). Systolic blood pressure is when the heart is in its contracting phase, this measurement represents the maximum amount of pressure in the arteries (Shahoud *et al.,* 2023). Normal systolic values should be 120 or below (Lüscher, 2018). There was a statistical significance between young healthy females and older healthy females, as well as between the young healthy females and the breast cancer survivors. With no statistical significance being detected between the older healthy females and breast cancer survivors. This may be again due to the same reasons discussed above in the systolic blood pressure due to them being directly linked. Additionally, due to there not being any real difference between the groups it is not possible to distinguish the age-related changes from the cancer related changes. It has also been suggested that breast cancer survivors are at a higher risk of developing high blood pressure compared to females who have never been treated for breast cancer (Kwan *et al.,* 2022).

Certain chemotherapy drugs can cause vasoconstriction, and this increases peripheral resistance, therefore causing high blood pressure (Thomas, 2017). Chemotherapy drugs can also have a direct effect on endothelial function, sympathetic activity, renin-angiotensin system activity and nephrotoxicity (Cohen *et al.,* 2019). The renin-angiotensin system is the regulator of blood volume, electrolyte balance and systemic vascular resistance in the body, and it is responsible for acute and chronic alterations of these above factors (Fountain *et al.,*

2023). Nephrotoxicity is when the kidneys are seriously damaged by toxicants, and therefore the kidneys cannot carry out their normal detoxification and excretion functions (Kim and Moon, 2012). So therefore, the higher blood pressure in the breast cancer survivors may be caused by the chemotherapy treatment they have received. However, it is difficult to distinguish between age related factors and cancer treatment related factors.

Average Heart Rate

Average heart rate was the highest (76 bpm ± 11) in breast cancer survivors when compared to older healthy females (71 bpm ± 14) and younger healthy females (74 bpm ± 16), however all average heart rate (bpm) data was classed as normal (between 60 – 100 bpm) (Olshansky *et al.,* 2023). The statistical tests did not find any statistical significance between the three groups.

It was anticipated that breast cancer patients may have higher resting heart rate as a high resting heart rate is associated with cancer. Treatment-naïve cancer patients have been found to have higher resting heart rates than average (Anker *et al.,* 2020). Additionally, several cancer treatments have been associated with resting sinus tachycardia. These include doxorubicin, idarubicin, paclitaxel and fluoropyrimidines and HER2 inhibitors (Sakellakis *et al.,* 2024). This may be attributed to a combination of different mechanisms causing the high resting heart rate in cancer patients including responding to increased metabolic demands, raised cardiac output states and pain (Sakellakis *et al.,* 2024; Anker *et al.,* 2020). Therefore, it is difficult to tell whether a high resting heart rate may be attributed by the body's compensatory mechanisms (mentioned above) or due to the cancer treatment itself, or a combination of these factors (Sakellakis *et al.,* 2024; Anker *et al.,* 2020). Suggesting that medical professionals should consider recording resting heart rate before cancer treatment

starts to allow for a baseline measurement. This would allow further investigation into these interactions.

Average Body Mass Index

Average BMI was classed as high (29.82 ± 3.74) in breast cancer survivors as the normal range of BMI should be between 18.5 – 24.9 (Weir and Jan, 2019). Young healthy females and older healthy females BMI average was classed as normal with young healthy females being 22.09 ± 3.51 and older healthy females being 24.73 ± 2.99. There was statistical significance between young healthy females and breast cancer survivors as well as between older healthy females and breast cancer survivors. This may be due to breast cancer survivors having a high BMI from the side effects during/after treatment (Ee *et al.,* 2023; Vance *et al.,* 2010). For example, breast cancer survivors may have been less able to exercise due to stress factors such as mental health, or not feeling educated enough of how much exercise they can do during cancer treatment. Lastly cancer treatment may have had a tiring effect on the individual throughout treatment, so therefore, little or no exercise or physical activity took place (Vance *et al.,* 2010). There is also evidence to suggest that having a high BMI may contribute to the development of cancer. It is suggested that every 5 kg/m^2 increase in BMI corresponds to a 2 % increase in breast cancer risk in women (Liu *et al.,* 2018). However, it is important to note that cancer is a multifactorial disease, meaning that it can develop and be caused by multiple factors, both genetic and environmental factors (Scrivo *et al.,* 2011). Hormone therapy (Tamoxifen) has also been associated with increases in BMI in women who have had breast cancer (Ee *et al.,* 2023).

Overall Interpretations

Overall, the main pattern that has been identified is that breast cancer survivors have the most negative result, followed by older healthy females and then younger healthy females. As seen

in PSV (cm/s), IMT (cm), systolic blood pressure (bp), diastolic blood pressure (bp), BMI (kg/cm^2), and the diameter of the CCA in longitudinal (cm) and transverse view (cm). Though not all of these results have been of significance in many cases, such as PSV, systolic and diastolic blood pressure. This is likely due to the small sample size, so a suggested pattern of data was identified rather than a statistical significance. However, a difference was still identified with breast cancer survivors having the most negative results. Therefore, this means that the ultrasound is still a viable method to use to identify cardiotoxicity, however more work is needed but early data suggests that this could be a viable new method of measuring cardiotoxicity in breast cancer survivors.

Chapter 6 – Limitations

6.1 Limitations

This study only had 10 participants for each group, so therefore this was a limitation to this study. A small sample size meant that differences between groups were not as easily detected unless they were very large differences between those groups. Participants were not age matched in this study and the breast cancer survivors were of any age, so this did not allow to distinguish what could have been due to age or cancer treatment. However, part of the purpose of this project was a pilot study to determine the type of sample and study design needed to investigate ultrasound to be able to successfully detect differences between the groups and potential cardiotoxicity in breast cancer survivors.

Further limitations of this study were that none of the participants had a confirmed cardiotoxicity diagnosis. This meant that any results that were collected could not be as easily confirmed as definitely cardiotoxicity. Each breast cancer survivor had different treatment combinations for the treatment of their breast cancer and durations of treatment, which could have influenced results. As some specific chemotherapy drugs are known to have a more damaging effect on the heart when compared to other chemotherapy drugs. The different duration of treatments may have meant that some participants could have been affected more severely than others due to a longer exposure or higher dose of treatment. Due to the heterogeneity of the breast cancer survivors, some breast cancer survivors had finished their breast cancer treatment earlier and for longer than others, which may have influenced the measurements that were taken. This meant that reversible cardiotoxicity (type II) could have been detected in some of the breast cancer survivors, and that it was not permanent cardiotoxicity (type I).

Recruitment for breast cancer survivors in this study was from the community rather than the NHS. Therefore, due to it being in the community it made it harder to recruit breast cancer survivors. However, it must be noted that this was a pilot study completed in one year, so recruitment had to be from the community due to the long ethics process for the NHS recruitment. The method of ultrasound used in this study measured the carotid artery and not directly the heart itself. So, measures could have detected other factors/issues not relating to the heart or cancer treatment-related heart damage. There is also no other comparison to any other measures of cardiotoxicity, so there is no baseline measures or normative measures to compare what is normal and what is classed as cardiotoxicity. However, this is due to the method of carotid ultrasound being a completely new concept to detect cardiotoxicity in breast cancer survivors. Lastly, there was no control for physical activity levels and diet, so therefore measures could have been influenced by other factors such as fitness level or diet.

Chapter 7 – Future Recommendations

7.1 Future Recommendations

Since this was a pilot study, it presents a variety of important information upon which to build future work. Future recommendations include completing this study on a larger scale with a larger number of participants for each group. This would allow for any differences between groups to be easily identified. Age matching participants from each group is also recommended to ensure that age is not the reason why there may be any differences of results. Additionally, if controls are age matched statistical precision may be improved to ensure roughly equal numbers of cases and controls are in each age group. Young and older breast cancer survivors results also need to be investigated and compared to understand the relationship of age on the measures.

Breast cancer survivors with a confirmed cardiotoxicity diagnosis would also make it easier to confirm the reliability of the carotid ultrasound method. This would help confirm the link between the measures (PSV, EDV, IMT etc.) and cardiotoxicity. Breast cancer survivors with the same treatment combinations would also make it easier to identify cardiotoxicity and if the new method works. This is due to some chemotherapy drugs having more of a damaging effect on the cardiovascular system such as anthracyclines (Sandoo *et al.,* 2014). So therefore, by looking at same treatment combinations it would also allow investigation into the chemotherapy drug itself and its interaction with the cardiovascular system.

A control of physical activity and diet would also be good to incorporate for future studies, to ensure there are no other factors influencing blood flow in the CCA such as increased physical activity levels or increased salt intake. Cardiovascular heath of breast cancer survivors with and without cardiotoxicity should also be compared to two non-malignant

groups. With female participants age-matched to the breast cancer survivors, and female participants under the age of 30. With the four groups being assessed for a variety of descriptive and cardiovascular related risk factors as well as cardiac and arterial-specific measures to create a comprehensive cardiovascular profile of the population groups. This will allow a comparison of measures between groups with the carotid ultrasound.

Chapter 8 – Conclusion

8.1. Conclusion

Overall, this study has shown that ultrasound could be a useful tool to investigate cardiotoxicity in breast cancer survivors. Ultrasound was used to measure PSV, EDV, IMT, longitudinal and transverse view of the CCA in young healthy females, older healthy females and female breast cancer survivors. PSV and IMT may be important markers of cardiovascular health in breast cancer survivors. A lower PSV and higher IMT may indicate that heart function is reduced in breast cancer survivors. This is due to research stating that a lower PSV and a higher IMT is indicative of reduced cardiovascular function and an increased risk of cardiovascular disease and events such as myocardial infarctions and strokes (Bendick, 2014; Coll and Feinstein, 2008).

It is also important to note that using ultrasound this way has never been explored before in research and is a completely new concept of detecting cardiotoxicity in breast cancer survivors. Despite the significant limitations of the current study, some significant differences were successfully detected between young healthy females, older healthy females and breast cancer survivors, such as PSV mentioned above. This means that with further investigation and development, ultrasound may be a useful tool to investigate cardiotoxicity.

Appendices

Appendix 1

Durham University
Department of Sport and Exercise Sciences

Inspiring the extraordinary

General Health Screening Questionnaire

For participation in physical activity and related activities within the Department of Sport and Exercise Sciences, Durham University

Please read all the questions below carefully and answer each one honestly: check YES or NO.	**YES**	**NO**
1) Has your doctor ever said that you have a heart condition **OR** high blood pressure?	☐	☐
2) Do you feel pain in your chest at rest, during your daily activities of living, **OR** when you do physical activity?	☐	☐
3) Do you lose balance because of dizziness **OR** have you lost consciousness in the last 12 months? *Please answer **NO** if your dizziness was associated with over-breathing (including during vigorous exercise*).	☐	☐
4) Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)? Please list condition(s) here: _____	☐	☐
5) Are you currently taking prescribed medications for a chronic condition? Please list condition(s) and medication(s) here: _____ _____	☐	☐
6) Are you pregnant or think you might be pregnant?	☐	☐
7) Are you breastfeeding?	☐	☐
8) Do you have any allergies? Please list allergies here: _____	☐	☐
9) Do you have any known infectious and / blood-borne disease(s)? Please list disease(s) here: _____	☐	☐
10) Do you take the contraceptive pill? If yes, please state which one:	☐	☐

Appendix 2

Breast Cancer Survivors Questionnaire

1. When were you diagnosed with Breast Cancer? Please include date, month, and year if possible.	
2. What treatment/s were you given?	
3. When did the doctor class you as officially cancer free? Please include date, month, and year if possible.	
4. Has it been one year or more since you had chemotherapy and/or radiotherapy treatment?	
5. When was the last dose of chemotherapy and/or radiotherapy treatment? Please include date, month, and year if possible.	
6. What stage of cancer were you diagnosed with?	

Appendix 3

Durham University

Incidental Findings and GP Referral Form

You are being sent this form because you recently participated in a research study in the Department of Sport and Exercise Sciences at Durham University. During this research study, we noticed that at least one of your measurements was outside the typical range of what we would expect. This is **NOT** a diagnostic test, but based on the outcomes of this assessment, we would recommend that you follow up with your GP. If you have any questions or concerns, please do not hesitate to contact us. Your GP is also welcome to contact us for any additional information they may require.

Date Form Prepared		**Date of Tests**	
Participant Name			
Study Name	Peripheral blood flow in the carotid artery at rest to investigate cardiovascular health in breast cancer survivors: A feasibility study.		
Study Principal Investigator	Katie Di Sebastiano, PhD Assistant Professor, Department of Sport and Exercise Sciences		
Contact Information	Email: kathleen.di-sebastiano@durham.ac.uk Phone: 0441913341477		
Researcher	Chelsea Penny		

Description of the Test Conducted:

Findings of the Test:

Expected Results:

Appendix 4

Clinical Characteristics of the Breast Cancer Survivors

	When were you diagnosed with Breast Cancer? Please include date, month, and year if possible.	**What treatment/s were you given?**	**When did the doctor class you as officially cancer free? Please include date, month, and year if possible.**	**Has it been one year or more since you had chemotherapy and/or radiotherapy treatment?**	**When was the last dose of chemotherapy and/or radiotherapy treatment? Please include date, month, and year if possible.**	**What stage of cancer were you diagnosed with?**
1BCS	21.01.2014	1) Lumpectomy and removal of lymph nodes 2) 6 rounds of FEC-T chemotherapy 3) 15 rounds of radiotherapy 4) Tamoxifen tablets for 5 years	2019 after 5 annual mammograms, none of which showed a recurrance.	Yes	Chemotherapy: 6 August 2014 Radiotherapy: approx. 23rd September 2014	Grade 2 stage 2
2BCS	31 December 2021	Immediate letrazole 2.5mg daily 7 April 2022 Mastectomy and immediate DIEP reconstruction. Onco DX score 12 Letrazole to be continued for 10 years	20 May 2022 (date told clear margins and no need for radio or chemotherapy	N/A	N/A	T2N0M0

3BCS	March 18 2022	Surgery April 16 2022 - Right breast wide local excision and sentinel lymph node biopsy May 28 2022 – Further wider excision. Hormone Therapy: Letrozole 2.5mg daily started September 12 2022	Mammogram April 5 2023 Letter received April 26 2023 - saying it showed no evidence of cancer. Mammogram will be repeated April 2024	Yes	Radiotherapy – August 1 2022	Stage 1
4BCS	20 February 2019	Surgery + radiotherapy + hormone blocking medication	-?	Yes	17 September 2019	B5a high grade ductal carcinoma
5BCS	1 June 2020	6 x FEC-T chemotherapy(3FEC + 3T) 5 x radiotherapy, 6 x zoledronic acid Letrozole for 5-10 years	25 June 2020 when they got clear margins	Yes	Last chemo 26 Nov 2020 Last radiotherapy 5 Jan 2021	Wasn't given a stage just a grade which was grade 3

6BCS	March 2018	Radiotherapy Bone infusions Letrezole Calcium vitamin d	I have not had the conversation with the doctor I had a 5 year review and need to continue tablets with 2 year bone checks	Yes 2018	11th June 2018	Stage 1 with lymph spread
7BCS	12th Jan 2020	Lumpectomy – Jan 2020 Radiotherapy – March 2020	Currently prescribed letrozole 2.5 mg Not yet	Yes	March/April 2020	Stage 2
8BCS	16th October 2003	Lumpectomy, Chemotherapy, Radiotherapy	I was told in December 2003 that they had removed all the cancer but I didn't get an official cancer free diagnosis. I was under the hospital for 10	Yes	Chemotherapy - 21st April 2004 Radiotherapy - 1st July 2004	I think it was stage 3 but am not certain as I can't fine any notes

			years after the operation.			
9BCS	28th June 2018	Surgery-wide local excision & sentinel lymph node biopsy on 20th July 2018 Radiotherapy & Letrozole for 5years	16th August 2023	Yes	Last radiotherapy date 9th October 2018	Grade 1
10BCS	September 2020	Chemotherapy x 8 (Nov20-Mar21) Radiotherapy x 15 (Jun/Jul21) Kadcyla x 14 (Aug21-Jul22)	Not been given this (yet)	Yes	Chemo – 30/03/21 Radiotherapy – 9/7/21	Left breast carcinoma T2, N1, ER positive, HER2 positive

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