ECHOCARDIOGRAPHIC MEASUREMENT OF CARDIAC FUNCTION IN BREAST CANCER PATIENTS TREATED WITH ANTHRACYCLINE CHEMOTHERAPY

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A thesis submitted in fulfillment of the requirements for the degree of

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DECLARATION

This thesis is an account of original research undertaken from 2008 to 2013 while a student at the University of Sydney. I am primarily responsible for the design, execution, analysis and reporting of the research; although it was necessarily undertaken with the assistance of others, who are duly acknowledged in the thesis. To the best of my knowledge, all references to other published work contained in the thesis are correct, and no part of the thesis has been submitted for any other degree.

Paul Warren Stoodley

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I have many people to thank - more that I can acknowledge here. However, there are certain people to whom I am particularly indebted, and must acknowledge. Without their help this thesis would not have been possible. I wish to express my gratitude to:

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ABSTRACT

INTRODUCTION

Anthracycline chemotherapy is the cornerstone of breast cancer treatment. Anthracyclines possess potent anti-tumor properties, and their benefits are confirmed by a considerable body of evidence. However, their efficacy is undermined by dose dependent cardiotoxicity that mandates close monitoring of cardiac function: the method of choice for monitoring cardiac function is transthoracic echocardiography.

Resting left ventricular (LV) ejection fraction (LVEF) is the key echocardiographic parameter used to monitor anthracycline-induced cardiotoxicity. However, LVEF is a coarse measure of systolic function with numerous limitations that constrain its use for monitoring cardiotoxicity. The recent development of a novel echocardiographic technique, myocardial strain imaging, may provide a more sensitive, accurate and reproducible measurement of LV function and therefore improve measures aimed at countering cardiotoxicity. The aim of this research was to investigate myocardial strain imaging by comparison with LVEF, for potentially earlier detection of LV dysfunction, in breast cancer patients treated with anthracyclines.

METHOD

Anthracycline naïve breast cancer patients were prospectively studied; 78 in the short-term over 3 months, and 50 in the intermediate term over 12 months. All patients were treated with standard regimens containing anthracyclines. Echocardiograms were performed at 4 time points; 1) within 7 days before commencing chemotherapy, 2) within 7 days of completing chemotherapy, 3) 6 months after, and 4) 12 months after chemotherapy. The echocardiographic images, together with other relevant clinical data acquired were analyzed. Two-dimensional (2D) LVEF (measured by Simpson's method), and myocardial strain imaging (measured by 2D speckle tracking echocardiography (2DSTE)) were most thoroughly investigated.

RESULTS

Global and regional longitudinal LV systolic strain was significantly reduced in the short-term; global longitudinal strain decreased from -17.7% to -16.3% ($p < 0.01$) with 48% of global measurements reduced by >10%. In contrast, no reduction in LVEF >10% after chemotherapy was

observed. Conventional Doppler, tissue Doppler and myocardial strain imaging measurements of diastolic function were also altered within 7 days of completing chemotherapy. The changes in diastolic function were associated with reduced systolic function - reduced early diastolic strain rate (E-Sr) being most strongly predicted by reduced post chemotherapy systolic strain.

In the intermediate-term, systolic function remained significantly reduced 6 months after treatment, and the reduced systolic strain 7 days after and 6 months after treatment occurred non-uniformly within the LV. In the majority of participants, LV dysfunction was noted to be transient, with strain values returning to normal by 12 months. Mean global longitudinal systolic strain values at the 4 time points were: -19.0% ± 2.3%, -17.5% ± 2.3%, -18.2% ± 2.2%, -19.1% ± 1.9%. Persistently reduced systolic strain 12 months after anthracyclines was observed in 16% of participants, and was associated with significantly higher cumulative anthracycline doses.

CONCLUSION

Left ventricular systolic and diastolic dysfunction were detected using myocardial strain imaging within 7 days of completion of anthracycline chemotherapy, without a similar discernible change in LVEF. In the majority of participants, LV systolic dysfunction was noted to be transient, with strain values returning to normal 12 months after treatment. However, LV systolic strain was persistently reduced in 16% of participants 12 months after anthracyclines, and was associated with significantly higher cumulative anthracycline doses.

Myocardial strain imaging is more sensitive than LVEF for the early detection and intermediate term monitoring of LV systolic function following anthracycline chemotherapy. Myocardial strain imaging may aid in the identification of those with preclinical LV dysfunction, and in the development of improved monitoring protocols.

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ABBREVIATIONS

The following is a list of abbreviated terms used throughout the thesis. All terms are written in full the first time they appear each chapter (with their abbreviation shown in parenthesis); the abbreviation is then used for the remainder of the chapter. (Abbreviated terms used less than three times in the thesis are not included in the list).

A′	late peak diastolic tissue velocity	HER2/ neu	human epidermal growth factor receptor 2
A wave	late peak diastolic filling velocity	IHD	ischemic heart disease
ACH	anthracycline chemotherapy	LA	left atrium
ACEI	angiotensin-converting-enzyme inhibitors	LPSS	longitudinal peak systolic strain
A-Sr	late peak strain rate	LV	left ventricular
CAD	coronary artery disease	LVEDV	left ventricular end diastolic volume
CMRI	cardiac magnetic resonance imaging	LVESV	left ventricular end systolic volume
CO	cardiac output	MHz	mega-Hertz
CRCD	chemotherapy related cardiac dysfunction	M-mode	motion mode
CSA	cross sectional area	RT	radiation therapy
Ds	diastolic strain	S′	peak systolic tissue velocity
DNA	deoxyribonucleic acid	SD	standard deviation
DOX	doxorubicin	SV	stroke volume
E′	early peak diastolic tissue velocity	TDI	tissue Doppler imaging
E wave	early peak diastolic filling velocity	TTE	transthoracic echocardiography
E-Sr	early peak tissue velocity	3DE	three-dimensional echocardiography
ECG	electrocardiograph	2D	two-dimensional
EPI	epirubicin	2DSTE	two-dimensional speckle tracking echocardiography
GLS	global longitudinal strain	VTI	velocity time integral
Hz	Hertz		

PUBLICATIONS AND CONFERENCE PROCEEDINGS

The following are lists of publications and abstracts arising from this research.

JOURNAL PUBLICATIONS

Paul W Stoodley, David AB Richards, Anita Boyd, Rina Hui, Paul R Harnett, Steven R Meikle, Karen Byth, Kirsty Stuart, Jillian L Clarke, Liza Thomas, 'Left ventricular systolic function in HER2/neu negative breast cancer patients treated with anthracycline chemotherapy: a comparative analysis of LVEF and myocardial strain imaging over 12 months' ***European Journal of Cancer*** [In Press] 2013 doi:10.1016/j.ejca.2013.06.046

Paul Stoodley, David Richards, Anita Boyd, Rina Hui, Paul Harnett, Steven Meikle, Jillian Clarke, Liza Thomas, 'Altered left ventricular longitudinal diastolic function correlates with reduced systolic function immediately after anthracycline chemotherapy' manuscript reference number: ***European Heart Journal – Cardiovascular imaging***, 2013 March; 14 (3): 228-34, Epub date 12 July 2012

Paul Stoodley, David Tanous, David Richards, Steve Meikle, Jillian Clarke, Rina Hui, Liza Thomas. 'Trastuzumab induced cardiotoxicity: the role of 2D myocardial strain imaging in diagnosis and management' ***Echocardiography***, 2012 July, 29 (6): E137-E140

Paul Stoodley, David Richards, Rina Hui, Anita Boyd, Paul Harnett, Steven Meikle, Jillian Clarke, Liza Thomas, 'Two-dimensional myocardial strain imaging detects changes in left ventricular systolic function immediately after anthracycline chemotherapy' ***European Journal of Echocardiography***, 2011; 12 (12): 945-952

Paul W. Stoodley, David A.B. Richards, Steve R. Meikle, Jillian Clarke, Rina Hui and Liza Thomas, 'The potential role of echocardiographic strain imaging for evaluating cardiotoxicity due to cancer therapy' ***Heart, Lung & Circulation***, 2011 January; 20 (1): 3-9

ABSTRACT PUBLICATIONS (CONFERENCE PROCEEDINGS)

P. Stoodley, A Boyd, P Harnett, D Richards, S.Meikle, J.Clarke, R Hui, L.Thomas 'Evaluation of left ventricular systolic function in the intermediate term after anthracycline chemotherapy: a comparison of LVEF and global longitudinal strain' ***Heart, Lung & Circulation*** 2012; 21 (supplement 1): page S202

P. Stoodley, A Boyd, P Harnett, D Richards, S.Meikle, J.Clarke, R Hui, L.Thomas 'Altered LV diastolic function early after anthracycline chemotherapy' ***Heart, Lung & Circulation*** 2011 Vol. 20 (supplement 2) page s158

P. Stoodley, A Boyd, P Harnett, D Richards, S.Meikle, J.Clarke, R Hui, L.Thomas 'Myocardial strain imaging detects early changes in global left ventricular systolic function after anthracycline chemotherapy' ***European Heart Journal*** 2011 32 (abstract supplement): page 4684

P. Stoodley, A Boyd, P Harnett, D Richards, S.Meikle, J.Clarke, R Hui, L.Thomas 'Reduced segmental left ventricular systolic strain after anthracycline chemotherapy' ***European Heart Journa***l 2011 32 (abstract supplement): page 1449

P. Stoodley, D. Richards, S.Meikle, J.Clarke, L.Thomas 'Acute Effects of Anthracycline Therapy on Cardiac Function' ***Heart, Lung & Circulation*** 2010 Vol. 19 (supplement 2) page s162

PUBLICATION STATEMENT (FOR THESIS CHAPTER 3A)

Statement from co-authors confirming the authorship contribution of the PhD candidate:

As co-authors of the paper 'The potential role of echocardiographic strain imaging for evaluating cardiotoxicity due to cancer therapy', we confirm that Paul Warren Stoodley's contribution to the paper is consistent with him being named first author. In particular, the candidate's contribution to the following items should be noted:

- conception and research of the review
- writing and critical appraisal of the content

Signed: [redacted] Date: 30 Sep 13
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CHAPTER 1

INTRODUCTION

1.1 MOTIVATION AND AIM OF THE THESIS

Cancer survival rates have greatly improved in the last decade due to refined conventional treatments and the development of new therapies (Jemal et al., 2011). However, improved survival has come at a cost; significant side effects are often related to treatment - cardiotoxicity being among the most serious (Sawyer, Peng, Chen, Pentassuglia, & Lim, 2010). Anthracycline agents are a model case in point: anthracyclines have powerful anti-tumor properties that have been central in the improved life expectancy for victims of numerous cancers, especially breast cancer (Gianni et al., 2008). Unfortunately, the effectiveness of anthracyclines in breast cancer treatment is undermined by their adverse effects on non-malignant cells, including cardiac myocytes (Singal & Iliskovic, 1998; Singal, Li, Kumar, Danelisen, & Iliskovic, 2000).

The cardiotoxic potential of anthracycline chemotherapy means that the focus of breast cancer treatment has been recast. As treatment most often involves anthracyclines, the aim is no longer to simply overcome malignancy: treatment now involves close monitoring of cardiac function before, during and after their use (Bird & Swain, 2008). The method of choice for monitoring cardiotoxicity in most centers is transthoracic echocardiography, the most popular imaging method for measuring cardiac anatomy and function (Bird & Swain, 2008; Yu, Sanderson, Marwick, & Oh, 2007). Although echocardiography has matured via extensive technical advances over six decades, there remains the need for further development. Encouragingly, novel innovations promise exciting further developments.

Currently, the most common echocardiographic method for assessing left ventricular (LV) systolic function is the measure of two-dimensional (2D) LV ejection fraction (LVEF), which provides the fraction of LV volume ejected in systole (see figure 1.1A) (Jurcut et al., 2008; Lang et al., 2005). It is widely acknowledged however, that LVEF has a number of significant limitations.

LVEF is dependent upon 2D image quality; if images are poor, accuracy is reduced (Otterstad, 2002). It also requires simplified assumptions about cardiac geometry that are used during measurement - if LV geometry is atypical or abnormal, the measurement is open to error (McGowan & Cleland, 2003). LVEF is also limited by the loading conditions on the heart (Bellenger et al., 2000). For example, an LVEF of 55% is normal in the majority of patients, however it is not normal in a patient with severe mitral valve regurgitation.

The recent development of a semi-automated echocardiographic technique, myocardial strain imaging, may provide more sensitive, accurate and reproducible measurements of LV systolic function (Marwick & Narula, 2009). Strain is a measure of myocardial deformation that represents the percentage change in length of a section of myocardium from its original length (see figure 1.1B) (Sengupta et al., 2007). Segmental LV dysfunction often precedes global dysfunction and is not captured by the LVEF; importantly, strain is able to measure both segmental and global myocardial deformation (Gorcsan & Tanaka, 2011). Furthermore, the muscular anatomy of the LV is complex; strain imaging is ideally suited to measuring LV function as it enables multiplane quantification of myocardial deformation (Geyer et al., 2010; Gorcsan & Tanaka, 2011).

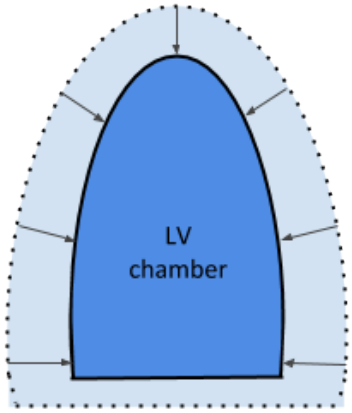

Figure 1.1A Illustration of the change in LV volume.

LVEF = (LVEDV - LVESV) / LVEDV

(where: LVEDV is the left ventricular end diastolic volume (indicated by the broken line), and LVESV is the left ventricular end systolic volume (indicated by the unbroken line)).

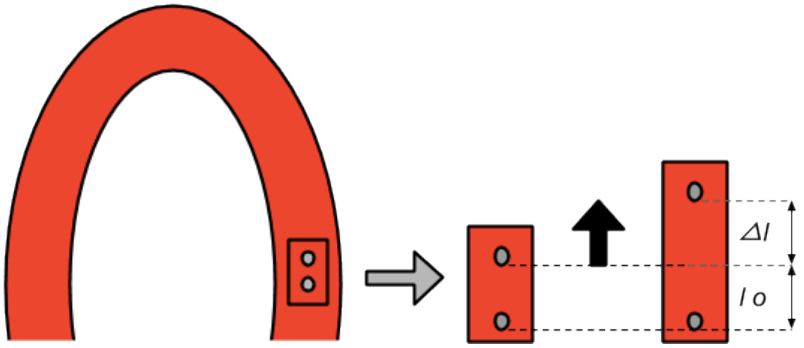

Figure 1.1B Illustration of myocardial strain measurement. An area of the LV myocardium is first highlighted and then shown in focus: **Strain (%) = △l / lo** *(where: △l is the change in length and lo is the original length).*

The value of myocardial strain imaging in routine clinical practice is yet to be fully realized. One area in which it may be of great benefit is in the collaboration between cardiology and oncology, when breast cancer involves anthracycline chemotherapy. Specific to anthracycline-induced cardiotoxicity, when a significant reduction in LVEF is demonstrated, functional deterioration can proceed quickly (Jurcut et al., 2008). Therefore, the early detection of LV dysfunction that enables the timely introduction of measures aimed at counteracting toxicity is important. Myocardial strain imaging, with its ability to measure systolic and diastolic function, may permit early detection of subclinical regional or global cardiotoxicity.

At the commencement of this thesis, data demonstrating that the early detection of cardiotoxicity using strain imaging may alter clinical prognosis was absent. Such data would facilitate modification of chemotherapy together with the introduction of therapy to minimize the impact of cardiotoxicity. The aim of this research was to investigate LV function in breast cancer patients treated with anthracyclines using both LVEF and myocardial strain imaging. To do so, images were acquired at specific time points; before, immediately after, and 6 and 12 months after chemotherapy. The echocardiographic images, together with other relevant data were analyzed, and the findings are presented in the following chapters of this thesis.

1.2 ORGANIZATION OF THE THESIS

Structured research was undertaken, with observations from the research published at appropriate times during the course of the study. Therefore, a large part of the thesis comprises peer reviewed published work. The chapters that contain published work are presented as they were accepted for publication, with the exception of minor differences in formatting (such as figure numbering and the referencing style) to ensure cohesion within the thesis. Referencing throughout the thesis is in American Psychological Association (APA) style.

The opening pages, introduction, background and concluding chapters, together with some supplementary material have been combined with the published work in order to unify the thesis. An outline of the structure of the thesis is provided in figure 1.2, which highlights the chapters that contain published work. A brief description of the contents of each chapter (apart from the present chapter) follows figure 1.2.

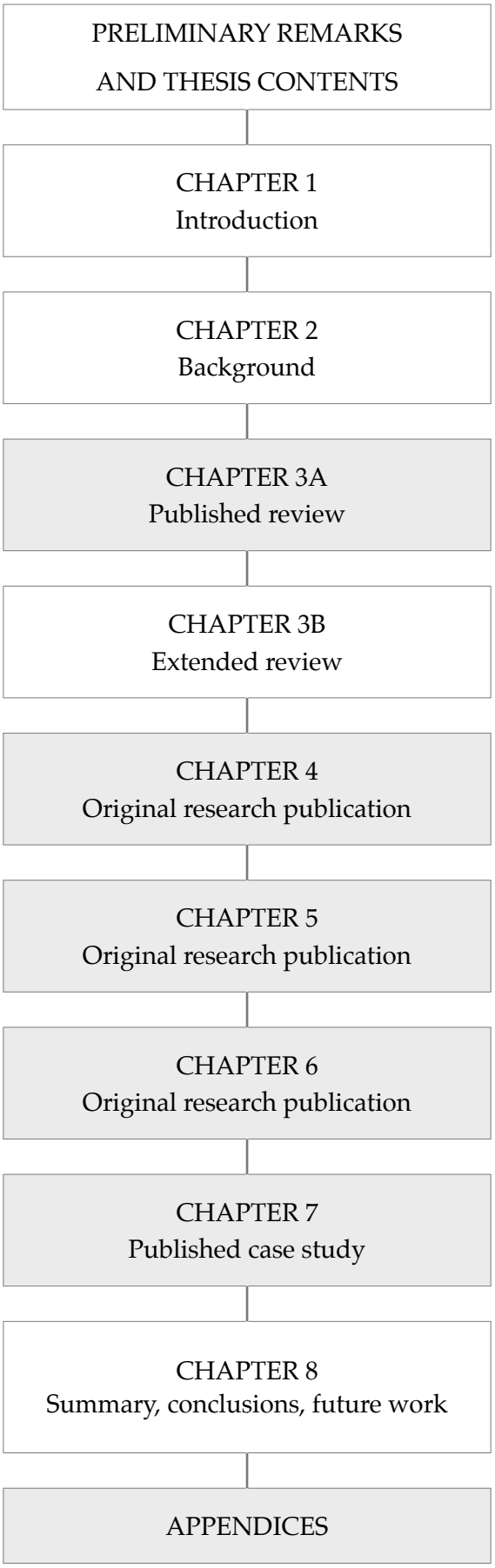

Figure 1.2 Diagram outlining the structure of the thesis with published work highlighted grey.

CHAPTER 2

The thesis is centered on echocardiography and anthracycline chemotherapy: essential background information about echocardiography is outlined in this chapter. More specifically, echocardiography has developed via a growing awareness of certain key principles for the observation and application of sound energy. This chapter begins with a description of a number of these key principles. Important advances in the development of ultrasound imaging, specifically echocardiography, are detailed next. Finally, developments in the measurement of LV function by echocardiography are discussed. (Detailed information about anthracyclines is given in chapter 3).

CHAPTER 3

This chapter consists of two parts. In part A, the published review article 'the potential role of echocardiographic strain imaging for evaluating cardiotoxicity due to cancer therapy' is presented. Due to the editorial limitations on the length and the number of references that could be used in the published review, and as 3 years have elapsed since the publication of the original review, additional information is presented in part B. With specific reference to anthracycline chemotherapy, the development of myocardial strain imaging, strain imaging methodology, and the potential benefit of utilizing strain imaging for evaluating cardiotoxicity, chapter 3 provides a comprehensive literature review.

CHAPTER 4

In this chapter, the published findings from analysis of LV systolic function using LVEF and strain imaging made immediately prior and immediately after anthracycline chemotherapy in the first fifty-two consecutive breast cancer patients are presented. Following a brief preamble, the chapter is presented as it was accepted for publication starting with the abstract, followed by the introduction, methods, results, conclusion and all references cited in the chapter.

CHAPTER 5

During the analysis of LV systolic function made immediately after anthracycline treatment, it was evident that an analysis of LV diastolic function would be a logical extension of the research. This is due to the complementary roles that both phases of the cardiac cycle play to ensure optimal cardiac performance. The published findings from analysis of diastolic function made immediately

before and immediately after anthracycline chemotherapy are presented in this chapter. As per chapter 4, this chapter is presented as it was accepted for publication and includes the abstract, introduction, methods, results, conclusion and a list of all references used in the chapter.

CHAPTER 6

The data from measurements of LV systolic function made during the extended follow-up of participants are presented in this chapter - data from 52 participants followed for 12 months with echocardiography at four time points. These results are therefore the necessary extension of the earlier findings, and provide longer-term measurements of LV systolic function after anthracyclines. In keeping with the two chapters immediately prior, the findings are presented as they were accepted for publication and include the abstract, introduction, methods, results, conclusion and a list of all references cited in the chapter.

CHAPTER 7

Anthracyclines are one of several agents used in the treatment of breast cancer. This chapter details the interesting and pertinent case of a 45-year-old female breast cancer patient who developed heart failure during treatment with trastuzumab which was administered following completion of anthracyclines. LVEF and myocardial strain measurements were made during her treatment for heart failure and during the reintroduction of trastuzumab therapy. As per the published article, the chapter includes an abstract, patient history, discussion and references.

CHAPTER 8

A summary of the entire thesis and a discussion of the key findings from the research begin this final chapter. Conclusions that can be drawn, limitations and questions that arise from the research are presented. Chapter 8 ends with a discussion of future research.

APPENDICES

Ethics approval details, participant information and consent forms, and conference proceedings (abstract publications) are included as appendix items.

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CHAPTER 2

BACKGROUND

Preamble

As described in chapter 1, the primary aim of this thesis is the evaluation of cardiac function in breast cancer patients treated with potentially cardiotoxic chemotherapy. More specifically, echocardiographic myocardial strain imaging was investigated, for potentially earlier detection of LV dysfunction, in breast cancer patients treated with anthracycline chemotherapy. As such, the thesis is centered on echocardiography and anthracycline chemotherapy.

Echocardiography uses ultrasound to create real-time images of the heart (J. K. Oh, Seward, J.B., Tajik, A.J., 2007). It has matured by way of extensive technical advances (outlined in this chapter), which continue to provide improvements to current applications and for the development of newer ones. Once cumbersome machines with suboptimal images have evolved into user-friendly and sophisticated diagnostic instruments (Newman & Rozycki, 1998). Echocardiography has a well established role in routine clinical cardiology, and is increasingly used for monitoring patients where concerns exist about the potential impact of treatment on cardiac function.

This chapter begins with a description of essential principles for the observation and application of ultrasound. Medical ultrasound imaging has benefited from a growing awareness of these principles via the collaboration of a variety of disciplines including engineering, physics, medicine and physiology (Newman & Rozycki, 1998). A description of certain milestones in the study of sound and important advances in the development of ultrasound imaging, specifically echocardiography, are then detailed. Finally, developments in the measurement of left ventricular systolic function by echocardiography (up to the introduction of myocardial strain imaging) are discussed. A detailed description of strain imaging and anthracycline chemotherapy is presented in chapter 3.

2.1 PHYSICAL PRINCIPLES OF ULTRASOUND

2.1.1 ULTRASOUND WAVES

Ultrasound is mechanical energy transmitted through an elastic medium (Armstrong & Ryan, 2010). Its propagation occurs via the vibration of particles within the medium, parallel to the line of propagation. The vibration of particles gives rise to alternating areas of higher pressure densely packed particles (areas of compression), and lower pressure areas of less densely packed particles (areas of rarefaction). Thus, ultrasound is a longitudinal, compressional pressure wave (see figure 2.1) (Kremkau, 2011).

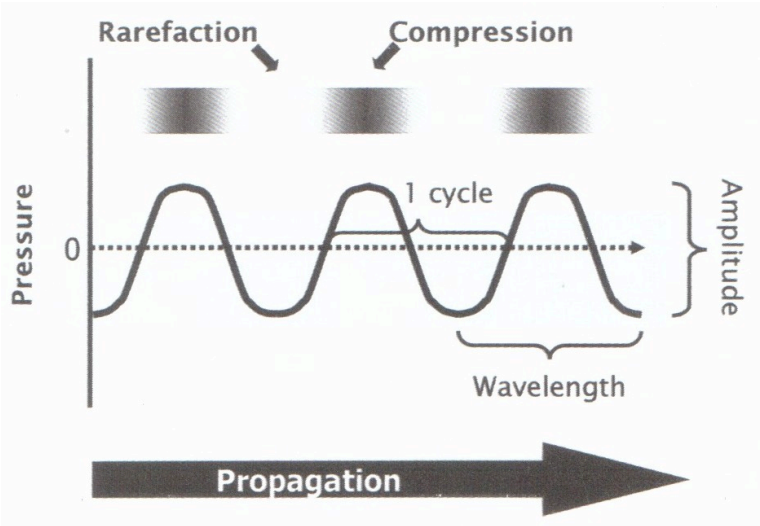


Figure 2.1. Illustration of the propagation of sound. Areas of high and low pressure are represented. Wavelength is determined by the frequency. The amplitude of the waveform is also shown. (Adapted from Armstrong & Ryan 2010).

The sum of one compression and one rarefaction of an ultrasound wave represents one cycle, and the distance between two similar points along the wave equals one wavelength (see figure 2.1). The frequency of the sound wave is the number of wavelengths per unit time. Therefore, wavelength and frequency are inversely related, and their product is the velocity of the sound wave (Kremkau, 2011):

$v = f \lambda$ (where: v = velocity, f = frequency, λ = wavelength)

The velocity at which the ultrasound wave travels is directly related to the stiffness of the medium. Ultrasound travels at a higher velocity through dense media (e.g. bone), and at a lower

velocity through less dense tissue (e.g. adipose tissue) (Armstrong & Ryan, 2010; Gent, 1997). The velocity of ultrasound within most soft tissue is very similar, approximately 1540 m/sec, which greatly aids the use of ultrasound for diagnostic imaging (Otto, 2004). Ultrasound wavelength is important in diagnostic imaging because: 1) image resolution is no greater than 1 to 2 wavelengths (usually about 1 mm) and 2) the depth of penetration is directly related to the wavelength - where shorter wavelengths penetrate a shorter distance than longer ones (Otto, 2004).

When sound waves move through air at an appropriate frequency, they are audible to the human ear (Armstrong & Ryan, 2010) (Jenkins, 2010). Ultrasound is the portion of the sound spectrum whose frequencies are above the audible limit of human hearing, that is those greater than 20,000 cycles per second (20,000 Hz) (Butler, 2009). The frequencies used in diagnostic ultrasound imaging are between 1 million and 20 million Hertz (1 - 20MHz) (Otto, 2004).

2.1.2 INTERACTION OF ULTRASOUND AND TISSUE

Reflection of sound waves is likely the most important interaction in ultrasound imaging (Gent, 1997). Reflection is the term used to describe the interaction between an incident ultrasound wave with tissue, that results in some or all of the wave reversing its direction (Bulter, 2009). The angle of approach of an incident sound wave and the angle of the reflected wave are always the same: reflection is described as being perpendicular (when the incident wave is related to the tissue by an angle of 90°) or oblique (when related by an angle other than 90°) (see figure 2.2)(Gent, 1997). The amount of ultrasound energy reflected at the interface of two media is proportional to the acoustic mismatch of the media - the greater the mismatch, the more energy is reflected (Gent, 1997). If the acoustic impedance of the two tissues is known, the percentage of the ultrasound beam reflected can be calculated (using the following formula (Cobbold, 2007)):

$$R = [Z_2 - Z_1 / Z_2 + Z_1]^2$$ *(where: R = the fraction of the ultrasound beam reflected, Z = the acoustic impedance of the tissue)*

As an ultrasound beam moves away from its source through tissue, it meets an array of big and small interfaces, each of which affects its transmission. These interactions can be broadly categorized as specular reflections or scattered reflections. Specular reflections are produced by reflectors that are larger than the wavelength of the ultrasound beam. This interaction results in a relatively angle-dependent reflection, whereby the optimal return of ultrasound waves back

toward the source, occurs when the ultrasound beam is perpendicular (90°) to the tissue (Armstrong & Ryan, 2010; Otto, 2004).

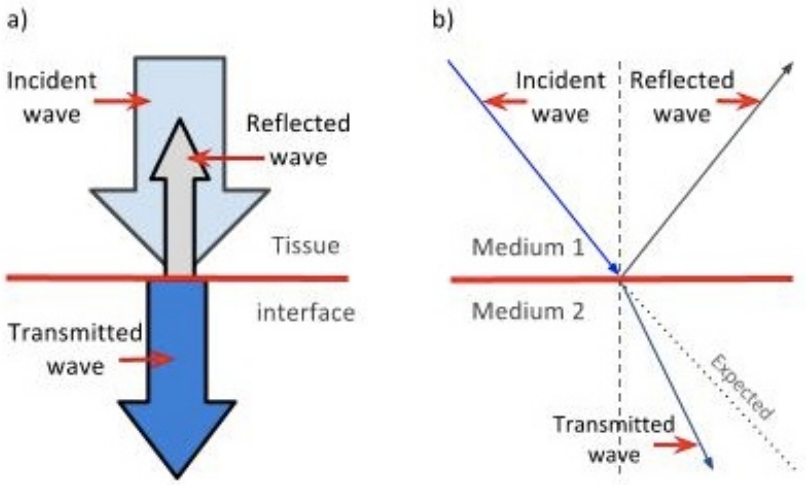


Figure 2.2 Depiction of: a) perpendicular reflection and transmission of an ultrasound wave at a tissue interface, and b) oblique reflection and transmission of an ultrasound wave at a tissue interface where the velocity of propagation in medium 1 is greater than medium 2.

In contrast, scattered reflections are produced by reflectors that are smaller than the ultrasound beam wavelength. The reflections that result are scattered in different directions, and the amount of energy that returns to the ultrasound transducer (at the source of the ultrasound beam) is significantly less than the energy that returns from specular reflectors. Scattering has important clinical significance; scattered reflections contribute to the visualisation of surfaces parallel to the ultrasound beam and add to the 'texture' of grey-scale images (Armstrong & Ryan, 2010). The term speckle is used to describe the tissue-ultrasound interaction that results from a large number of small reflectors, and this important interaction is discussed further in chapter 3 where speckle tracking echocardiography is described.

As scattering results in the reflection of ultrasound in different directions, it contributes to the attenuation (the decline) of the signal strength as it travels through tissue. Refraction, the deviation of an ultrasound wave as it passes through tissue, is another interaction that can enhance or compromise image formation (Gent, 1997; Otto, 2004). The most important factor related to the attenuation of the ultrasound beam is frequency, where lower ultrasound frequencies penetrate further into the tissue (attenuate less) than higher frequencies (Kremkau, 2011). In general, the attenuation of ultrasound in soft tissue observes the following rule (Gent, 1997):

Attenuation = 1dB / cm / MHz *(where dB = decibels, cm = centimeters, MHz = megahertz)*

Thus, techniques for improving image resolution (including increased transducer frequency) must be balanced with those for reducing attenuation (including reducing transducer frequency) to ensure optimal image quality.

2.2 VISUALIZING SOUND

Sound is mechanical energy that surrounds and informs nature. Sound waves with a frequency between 20Hz and 20kHz can be sensed by the human ear. An 18th century Italian scientist, Lazzaro Spalzani, is referred to as the father of ultrasound; he was the first to describe the use of sound in nature that is inaudible to the human ear by demonstrating that bats use reflected ultrasound to navigate in the dark (Krishnamoorthy, Sengupta, Gentile, & Khandheria, 2007).

In the early nineteenth century the Swiss physicist Jean-Daniel Colladon determined the speed of sound in water. His simple technique involved striking an underwater bell while simultaneously igniting gunpowder: the time between the sight of the gunpowder smoke and the sound of the bell and were recorded at a distance of 10 miles and used to make the calculation (which proved a very accurate measurement). This advancement provided a greater appreciation of underwater acoustics and sound transmission, and was central in the later development of SONAR (Sound Navigation and Ranging) (Newman & Rozycki, 1998).

In the middle of the nineteenth century, Swiss astronomer Christian Doppler, sought to explain how the observed frequencies of light waves are affected by the relative motion of the source and the detector (Goetz, 2010). After further development by contemporary scientists, it was discovered that the (Doppler) effect he sought to describe was applicable to sound as well as to light. The Doppler effect is used in many fields of science and medicine, including current ultrasound imaging (Newman & Rozycki, 1998). (In modern clinical echocardiography, the Doppler shift (the difference between received and transmitted frequencies) is most frequently used to measure the velocity of blood flow, and measured by the modified Doppler equation (Anderson, 2002):

$V = C (\pm \triangle f) / 2 f_o \cos \theta$ *(where: C = the speed of sound in soft tissue,* $\pm \triangle f$ *= the Doppler frequency shift (Hz),* f_o *= the transmitted frequency, cos* θ *= the angle between the ultrasound beam and blood flow))*

In the late nineteenth century, French brothers Pierre and Jacques Currie, made a discovery that was not immediately related to the study of sound energy, but one that would later prove very important. They observed that when pressure was applied to quartz crystals, an electric charge was generated in proportion to the force applied to it. This phenomenon was called Piezoelectricity (after the Greek word Piezo which means 'to press') and was essential in the development of the ultrasound transducer, and remains important for the transduction of mechanical into electrical energy (Newman & Rozycki, 1998).

Two historically important events are regarded as having provided significant motivation for the establishment of SONAR technology in the early 20th century. The first was the sinking of the RMS Titanic, the second was the quest for naval superiority in World War I (Newman & Rozycki, 1998). Furthermore, in the middle of the 20th century, reflected radio waves were used in World War II to detect enemy airplanes, a technology termed RADAR (Radio Detection and Ranging) (Newman & Rozycki, 1998).

2.3 THE DEVELOPMENT OF ECHOCARDIOGRAPHY

Echocardiography is the discipline that uses ultrasound to study the structure and function of the heart and great vessels (Armstrong & Ryan, 2010). Its has been described as the most important advance in diagnostic cardiology since the discovery of X-rays (Roelandt, 2000). The above mentioned milestone paved the way for the development of echocardiography, which in many aspects occurred together with developments in other disciplines that utilize ultrasound for diagnostic imaging. Echocardiography's relatively short but interesting history, is briefly described below.

2.3.1 THE BEGINNING

Motivated by the success of X-ray imaging in medicine, Austrian neurologist Karl Theo Dussik, was the first to attempt to utilize ultrasound for diagnosis in the early 1940s. He endeavored to outline the ventricles of the brain, but had limited success due to the highly reflective nature of the

skull bones (Krishnamoorthy et al., 2007). Shortly after, German physicist Wolfe Dieter Keidel, envisaged the use of ultrasound for recording the rhythmic volume variation of the heart. However, like Dussik, he met with significant technical difficulties and did not persevere in his efforts (Singh & Goyal, 2007).

In the early and mid 1940s, ultrasound was first used to determine the integrity of non-biological material (e.g. steel) at the University of Michigan, and researchers at the Naval Medical Research Institute in Maryland determined the speed of sound in soft biological tissue (Newman & Rozycki, 1998). Numerous technical advances occurred in the 1950's; Piezoelectricity was used by Hans Jaffe in ultrasound transducers, and techniques to improve the coupling of transducers to patients were developed by researchers in the U.S., Europe and Japan (Newman & Rozycki, 1998).

John Julian Wild and Douglas Howry are regarded as two of the most important early figures in ultrasound. Wild published measurements on the thickness of the bowel wall determined by ultrasound as early as 1950, and the identification of thigh and breast tumors in 1952 (Newman & Rozycki, 1998). In contrast, Howry was concerned with the applied theory of ultrasound and the development of equipment, rather than clinical applications. For example, Howry demonstrated that B-mode (brightness-mode) images display the most intense reflections from tissue interfaces that are perpendicular to the ultrasound beam (Newman & Rozycki, 1998).

Cardiologist Inge Edler and physicist Hellmuth Hertz are considered first among the pioneers of echocardiography. In the early 1950s, the Swedish duo undertook their first experiments using ultrasound to image cardiac anatomy and function. Their research provided important insights; they demonstrated that the interface between the muscular heart wall and its blood-filled chambers could be identified, that the thickness of the interventricular septum could be measured, and that mitral stenosis and mitral regurgitation could be differentiated with ultrasound (Edler & Lindstrom, 2004).

Edler and Hertz also developed a method of continuously recording echoes from moving cardiac structures. Echoes from nonmoving structures appeared as straight lines on film, while those from moving structures changed position in parallel with the motion of structures from which they were reflected (Edler & Lindstrom, 2004; Singh & Goyal, 2007). This significant development is now known as the motion mode (M-mode) technique, and is still utilized in modern echocardiography (see figure 2.3).

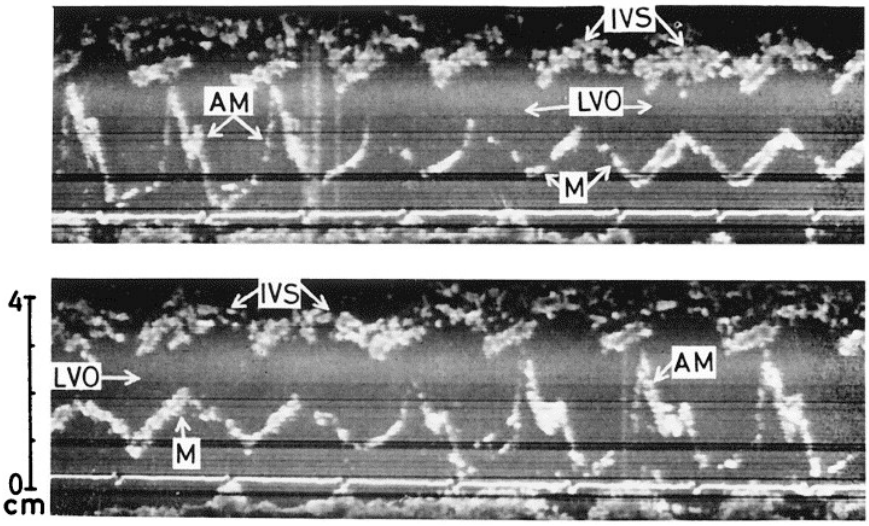


Figure 2.3 An early M-mode image published by Edler and Hertz: AM = anterior mitral leaflet, IVS = interventricular septum, LVO = left ventricular outflow tract, M = posterior wall of the LVO. (Adapted from Edler & Lindstrom, 2004).

While image acquisition was slow and display resolution was marginal, the 1960s was a decade during which ultrasound gained wider respect within the medical community. Real-time imaging was now seen as the way of the future; Hertz and Abserg published two-dimensional (2D) cardiac images (Asberg, 1967; Hertz, 1967), the first commercially available ultrasound machines was manufactured and used by Hoffman and Hollander in the late 1960s for gynecological imaging (Levi, 1997), and Nicholas Bom and his colleagues developed a linear scanner that made real-time imaging a reality (Armstrong & Ryan, 2010).

2.3.2 CONSOLIDATION

The more extensive use of echocardiography came in the 1960s (Feigenbaum, 1996). Harvey Feigenbaum, an American cardiologist, was captivated by echocardiography when he realized that a pericardial effusion could be imaged, and shortly after he made measurements of the LV using the M-mode technique (Feigenbaum, Popp, Chip, & Haine, 1968). While his initial work found limited approval, he was convinced of the potential of cardiac ultrasound; he initiated the first training program in cardiac ultrasound, and is credited with providing it with the name ‘echocardiography’ (Feigenbaum, 1996; Singh & Goyal, 2007).

Japanese researchers, including Shigeo Satomura, used Doppler technology, based on the change in frequency of reflected signals from moving structures, to examine blood flow within the

heart in the late 1950s (Feigenbaum, 1996). The principles and applications of pulse-wave and continuous-wave Doppler were developed in the 1970s, however the breakthrough in Doppler did not come until 1980, through research that demonstrated the value of Doppler technology in the assessment of aortic stenosis (Baker, Rubenstein, & Lorch, 1977; Feigenbaum, 1996; Hatle, Angelsen, & Tromsdal, 1980). Japanese researchers, including Ryozo Omoto, were also instrumental in the development of color Doppler imaging, which further advanced the haemodynamic assessment possible with echocardiography (Omoto et al., 1984). Color Doppler quickly became, and remains, an essential part of echocardiographic imaging (see figure 2.4).

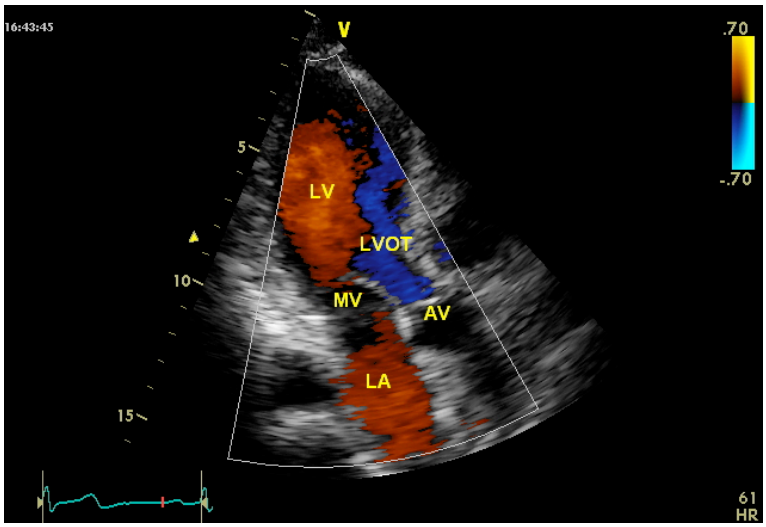


Figure 2.4 Apical long axis image with color Doppler. Blood flow travels toward the transducer (red) from the left atrium (LA), through the mitral valve (MV) and into the left ventricle (LV). Flow away from the transducer (blue) in the LV outflow tract (LVOT) does not go beyond the closed aortic valve (AV).

Real time 2D imaging in the clinical setting had been the goal of cardiologists and engineers worldwide, and became a reality in the late 1970s and early 1980s (Armstrong & Ryan, 2010). With this advance came vastly superior spatial resolution. Other developments in transducer technology also enhanced image quality; one notable example is harmonic imaging, which uses the relationship of reflected waves to the fundamental frequency of the ultrasound signal to exclude artifactual echoes (Belohlavek et al., 1998; Mulvagh, Foley, Belohlavek, & Seward, 1998). The use of contrast agents in echocardiography improved cardiac chamber visualization thereby improving diagnostic information, especially in patients with suboptimal images (Krishnamoorthy et al., 2007). This approach, which began with an incidental observation, is

currently most widely used for the detection of intra-cardiac shunts (Feigenbaum, 1996; Gramiak, Shah, & Kramer, 1969).

2.3.3 THE MODERN ERA

Tissue Doppler imaging (TDI) is a more recent echocardiographic development that has established its place in clinical echocardiography over the past decade. In contrast to other techniques that utilize Doppler to measure blood flow, in this case Doppler is used to measure tissue velocities (see figure 2.5). The TDI technique benefits from the fact that cardiac muscle moves at lower velocities than blood, but produce signals of a considerably higher amplitude, meaning that information regarding myocardial velocities can be obtained without contamination from blood-flow velocities (Krishnamoorthy et al., 2007).

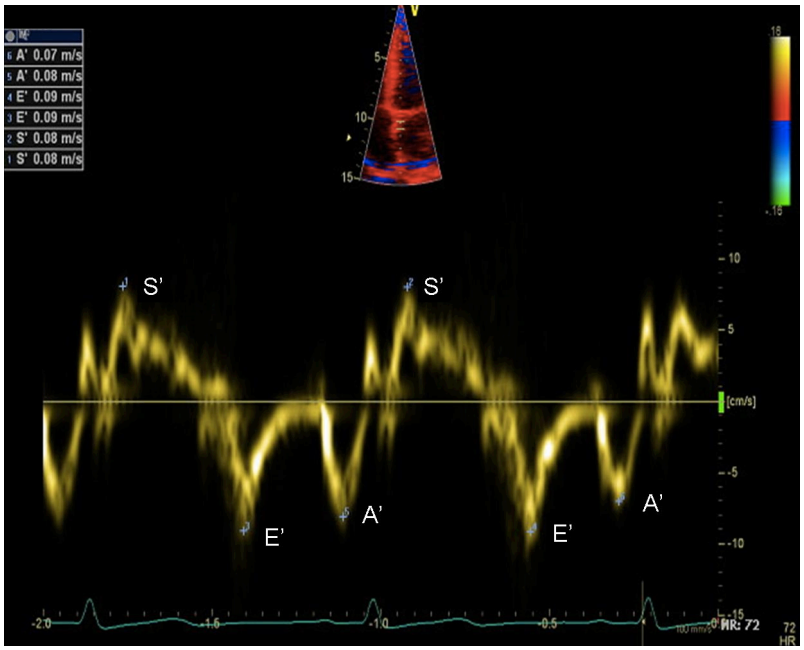


Figure 2.5 Tissue Doppler Imaging (TDI) of the mitral annulus from the apical 4 chamber view. S' represents the systolic myocardial velocity, the E' represents the early diastolic myocardial velocity, the A' represents the late diastolic myocardial velocity.

TDI is used to evaluate both LV systolic function (S' velocity) and diastolic function (E' and A' velocities) (see figure 2.5). However, its primary use is in the assessment of LV diastolic function, as it is particularly well suited to estimating LV relaxation in early diastole (E'). The ratio of the early transmitral filling velocity (E) to E' is a surrogate measure of the LV end diastolic pressure (Marwick, 2003). It has prognostic value in the assessment of systolic function post myocardial infarction and in heart failure patients, and it provides valuable information in patients

who have received resynchronization therapy (via an implantable dual chamber pacemaker) (Yu, Sanderson, Marwick, & Oh, 2007). However, like other Doppler techniques, TDI has a number of limitations; these include the need to ensure a parallel orientation between the ultrasound beam and the direction of myocardial motion and its inability to account for tethering from adjacent tissue (Geyer et al., 2010).

Improvements in transducer technology and computing power have been essential to the development of three-dimensional echocardiography (3DE). Most recently, the development of the matrix-array transducer (with its elements arranged in a rectangular grid) has made real-time 3DE a reality (Picard, Popp, & Weyman, 2008). 3DE has been shown to represent and quantify LV volume and function more accurately than single plane and 2D approaches (Siu et al., 1995). Yet, despite its many attractive features, 3DE is yet to become part of routine clinical echocardiography due to a number of limitations. Image quality in real-time 3DE is often poor (and the acquisition time for high quality images is considerable), and variations in cardiac rhythm and respiration are often difficult to redress (Picard et al., 2008).

2.4 MEASURING LEFT VENTRICULAR SYSTOLIC FUNCTION

From its inception, measuring LV systolic function has been an integral part of echocardiography. The first M-mode images, recorded by Edler and Hertz, displayed the movement of the posterior left ventricular (LV) wall (Edler & Lindstrom, 2004). Advancements in imaging technology have improved the measurement of systolic function considerably, however, the quest for a more accurate measurement of global and regional systolic function remains a major focus in echocardiography.

2.4.1 MOTION MODE (M-MODE) MEASUREMENTS

M-mode was the first method employed to measure LV systolic function, and was the technique of choice until the late 1970s (Picard et al., 2008). The M-mode method takes advantage of the change in LV internal dimensions in each cardiac cycle to measure stroke volume, and correlates favorably with angiography in normal shaped hearts (see figure 2.6). However, in conditions causing marked LV dilatation, or in those with significant regional wall motion abnormalities, the linear M-mode measurements are limited (Kronik, Slany, & Mosslacher, 1979; Teichholz, Kreulen, Herman, & Gorlin, 1976).

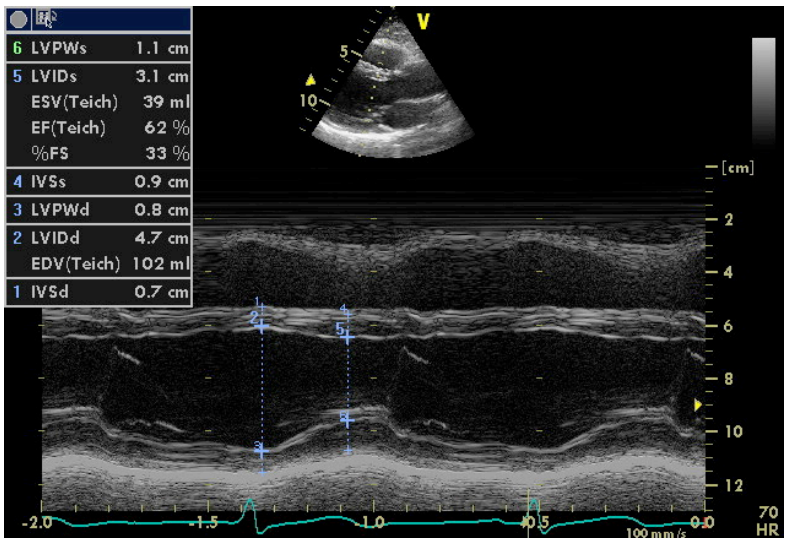


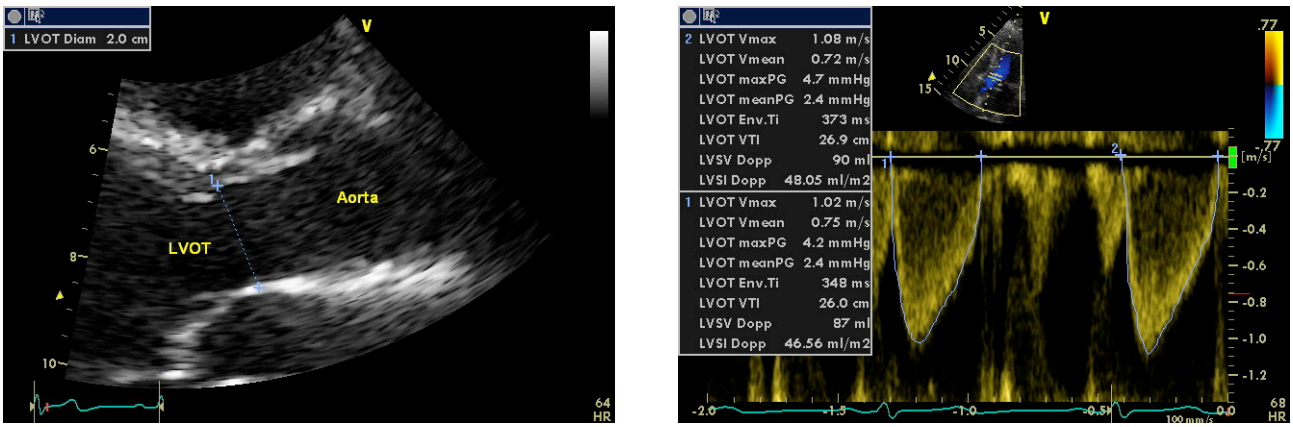
Figure 2.6 M-mode image and measurements of LV systolic function acquired from the parasternal long-axis view. 1 IVSd = interventricular septum (diastole), 2 LVIDd = LV internal dimension (diastole), 3 LVPWd = LV posterior wall (diastole), 4 IVSs = interventricular septum (systole), 5 = LVIDd = LV internal dimension (systole), 6 LVPWs LV posterior wall (systole).

2.4.2 DOPPLER MEASUREMENTS

The introduction of Doppler during echocardiography's infancy brought an additional means for measuring left ventricular function. Doppler enables the measurement of volumetric flow (based on the principle that flow is directly proportional to the cross-sectional area (CSA) of the chamber and the mean velocity of the moving fluid (see figure 2.7)) (Anderson, 2002; Armstrong & Ryan, 2010). Doppler allows indices like stroke volume and cardiac output to be measured using the following equations (Anderson, 2002):

> ***SV = CSA × VTI*** *(where: SV = stroke volume (cc), CSA = cross sectional area (cm^2), VTI = the distance a column of blood flows each stroke (the velocity time integral)*

> ***CO = SV × hr / 1000*** *(where: SV = stroke volume, hr = heart rate)*



A) B)

Figure 2.7 A and B Images and calculations used to measure stroke volume from the left ventricular outflow tract. A) LVOT measurement acquired from the parasternal short axis view, B) velocity time integral (VTI) recorded in the LVOT from the apical 5 chamber view.

Doppler measurements are an integral component of the majority of echocardiographic examinations, although they always involve multiple steps and are often limited by potential sources of error. The two most important sources of error shown in figure 2.7 are 1) in the determination of the CSA of the LVOT because the formula used to make the calculation assumes circular geometry of the LVOT (when in reality it is usually elliptical) and 2) the failure to optimize the Doppler angle (Armstrong & Ryan, 2010).

Another Doppler based method for the measurement of LV systolic function is the LV dP/dt. Using the spectral display of the mitral regurgitation jet, the rate at which the pressure increases within the ventricle can be measured. However, two important limitations mean that this technique has limited application; these are 1) increased LA pressure and 2) an inadequate Doppler signal (when there is poor alignment of the Doppler beam with the regurgitant jet direction, which is often the case with eccentric jets of regurgitation, or when there is insufficient mitral regurgitation) (Anderson, 2002).

2.4.3 TWO DIMENSIONAL (2D) MEASUREMENTS

The inception of real-time 2D echocardiography in the early 1980s brought superior spatial resolution for determining LV size and function (Armstrong & Ryan, 2010). Quantitative methods were soon introduced to aid 2D measurements. Initially, the single or biplane plane area length method was used to make LV volume and ejection fraction measurements. Recommendations and

normal values for this method were established, however its primary limitation, LV asymmetry, meant its application was limited (Anderson, 2002; Schiller et al., 1989). More recently, the biplane method of discs (Simpson's method) has been introduced, and it remains the method recommended by the American Society of Echocardiography and the European Association of Echocardiography (Lang et al., 2005), and is the most widely used quantitative method to assess LV systolic function in clinical practice.

Despite its wide use, it is generally acknowledged that LVEF has a number of significant limitations. LVEF is dependent upon image quality - poor images reduce its accuracy; and while developments such as higher frequency transducers and harmonic imaging have led to improved quality, this limitation remains (Bellenger et al., 2000; Malm, Frigstad, Sagberg, Larsson, & Skjaerpe, 2004; Otterstad, Froeland, St John Sutton, & Holme, 1997). Another major limitation is the simplified assumptions about cardiac geometry that are used during measurement - if LV geometry is atypical or abnormal, the measurement is open to error (McGowan & Cleland, 2003). LVEF is also limited by the loading conditions on the heart (Otterstad et al., 1997), which together with other factors, is described in greater detail in the following chapters of the thesis, where LVEF is contrasted with newer echocardiographic techniques.

Abnormalities in myocardial contractility occur when myocardial perfusion is impaired because of coronary artery disease. Echocardiography is the most widely used and practical technique for evaluating patients in this setting (J. K. Oh, Seward, J.B., Tajik, A.J., 2007). The wall motion score index (WMSI), which involves assigning a score to each region of the left ventricle based on contractility, is the qualitative method recommended for evaluating the extent of regional (segmental) wall motion abnormality (Schiller et al., 1989). While this method is subjective, it correlates closely with other methods used to estimate the size and site of the perfusion defect (J. K. Oh et al., 1996), however its application is largely confined to the evaluation of regional (segmental) LV wall motion.

2.4.4 TISSUE DOPPLER MEASUREMENTS

The M-mode, real-time 2D imaging, spectral and colour Doppler techniques detailed above, have significantly improved the analysis of LV systolic function. They allow important information related to changes in LV shape, LV volume, and haemodynamics (Marwick & Narula, 2009). However, the limitations described above indicate that these techniques are restricted in their

ability to measure myocardial mechanics. The introduction of TDI, a method capable of measuring tissue velocities, was an important advance in this regard.

TDI, outlined in section 2.3.3, is most widely used in the assessment of LV diastolic function. It is also used in the assessment of systolic function: mitral annular S' is a simple measurement to record, which represents the systolic myocardial velocity (see image 2.2) (Marwick, 2003). However, like other Doppler techniques, TDI has a number of limitations, which include the need to ensure a parallel orientation between the ultrasound beam and the direction of myocardial motion (Picard et al., 2008). Perhaps the most significant limitation in its use for measuring LV systolic function is its inability to account for tethering from adjacent tissue (Geyer et al., 2010).

2.4.5 MYOCARDIAL STRAIN IMAGING

The recent development of a semi-automated echocardiographic technique, myocardial strain imaging, may provide a more sensitive and accurate measurement of LV systolic function. Strain enables the measurement of both segmental (regional) and global myocardial deformation in multiple planes (Geyer et al., 2010). It has been shown to be of value in the identification of hypertrophic and infiltrative cardiomyopathies, and in the detection of subclinical dysfunction before a noticeable decrease in LVEF (Koyama & Falk, 2010; Serri et al., 2006; Weidemann et al., 2003).

Myocardial strain imaging is at the forefront of echocardiographic imaging technology, though its value in routine clinical practice is yet to be fully realized. Myocardial strain is detailed extensively in the remainder of the thesis. In the literature review its technical aspects are described, and in the subsequent chapters the results of research utilizing strain imaging for potentially earlier detection of LV dysfunction in breast cancer patients treated with anthracycline chemotherapy are presented.

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CHAPTER 3 (PART A)

LITERATURE REVIEW

Preamble

Echocardiography currently plays an important role in the surveillance of patients treated with potentially cardiotoxic anti-cancer agents. The present literature review describes this role, and outlines the potential benefits of myocardial strain imaging in this setting. The chapter is divided into two parts - A and B. In part A, the review article entitled 'The potential role of echocardiographic strain imaging for evaluating cardiotoxicity due to cancer therapy' is presented as accepted for publication (with only minor differences, such as figure numbering, to the published article to ensure continuity with the rest of the thesis). Due to the editorial constraints, the length of the review and the number of references included were limited, therefore an additional section (part B) has been added to the thesis. There is limited overlap of material in the two chapters, and together they provide a comprehensive literature review.

THE POTENTIAL ROLE OF ECHOCARDIOGRAPHIC STRAIN IMAGING FOR EVALUATING CARDIOTOXICITY DUE TO CANCER THERAPY

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ABSTRACT

Refinements to conventional treatment and the development of new therapies have led to significant improvements in cancer survival. Yet, many frontline cancer treatments continue to be hindered by their significant side effects, among which cardiotoxicity is particularly important. Therefore, the focus of cancer management has changed; treatment is no longer aimed solely at overcoming malignancy, but emphasizes early identification and treatment of potential side effects. In this regard, the cardiotoxic potential of certain anticancer agents mandate close monitoring of cardiac function, and the method of choice for monitoring is transthoracic echocardiography. While this method has its limitations, a newer echocardiographic technique called myocardial strain imaging has the potential to detect early sub-clinical changes in cardiac function due to cardiotoxicity. Strain analysis has been the subject of several recent studies to evaluate its potential in monitoring cardiotoxicity, and this article reviews the recent literature and explores the potential role of myocardial strain imaging in cancer management and avenues for future research.

3A.1 INTRODUCTION

Refinements to conventional treatment regimens and the development of new cancer therapies have led to significant improvements in cancer survival. For example, the five-year relative survival rate for breast cancer has risen from 75% in 1977 to 89% in 2004 and for leukemia and non-Hodgkin lymphoma from 42% in 1977 to 58% in 2004 ("Cancer Statistics 2009 Presentation," 2009). However, the efficacy of many frontline cancer treatments continues to be hindered by their significant side effects.

Thus, the focus of cancer management has changed; treatment is no longer aimed solely at overcoming malignancy, but emphasizes early identification and treatment of potential side effects. Specifically, the cardiotoxic potential of agents routinely used in the treatment of breast cancer, leukemia and non-Hodgkin lymphoma mandate close monitoring of cardiac function before, during, and after their use (Bird & Swain, 2008; Singal & Iliskovic, 1998).

Transthoracic echocardiography (TTE) is the method of choice for monitoring cardiac function in clinical practice (Jurcut et al., 2008a). Currently, evaluation of cardiac function by TTE involves measuring left ventricular ejection fraction (LVEF), a coarse measure of systolic function that has numerous limitations. Newer echocardiographic techniques have enhanced the capability to detect cardiotoxicity at an early stage; in this regard, myocardial strain imaging promises to be particularly useful. Strain imaging has been the subject of several recent studies to evaluate its potential in monitoring cardiotoxicity, and this article reviews the recent literature and explores the potential role of strain imaging in cancer management and avenues for future research.

3A.2 CHEMOTHERAPY AGENTS AND CARDIOTOXICITY

Anthracyclines were discovered four decades ago (Arcamone, Franceschi, Penco, & Selva, 1969), and remain among the most widely prescribed anti-cancer agents. They possess highly effective antineoplastic properties and are used to treat both adults and children; yet their efficacy is undermined by a potential life threatening cardiotoxicity (Lefrak, Pitha, Rosenheim, & Gottlieb, 1973; Shan, Lincoff, & Young, 1996; Singal, Li, Kumar, Danelisen, & Iliskovic, 2000).

Cardiomyopathy and heart failure from anthracyclines are dependent upon several treatment and patient related factors of which the cumulative administered dose is most critical. In adults the incidence of heart failure induced by doxorubicin (an anthracycline) varies from 4% to

5% at a cumulative dose of 500-550 mg/m^2, to 36% at a cumulative dose 600 mg/m^2 or more (Singal & Iliskovic, 1998; Swain, Whaley, & Ewer, 2003). Current treatment protocols pay careful attention to cumulative chemotherapy dosage with administration now by infusion rather than by bolus ("Anthracyclines and mitoxantrone - cumulative dose cardiac toxicity,," 2009). Furthermore, as previous anthracycline therapy as well as mediastinal irradiation increases the risk of cardiotoxicity (Swain et al., 2003), consideration of previous treatments is important.

Risks factors that predict cardiotoxicity, other than those inherent to anthracycline administration have been identified; they include the extremes of age (< 4 years and > 60 years), significant hypertension, female gender and malnutrition (Grenier & Lipshultz, 1998; Singal & Iliskovic, 1998). In patients with metastatic breast cancer, poor performance status and a higher body weight are factors with significant predictive value (Dranitsaris et al., 2008). While not definitively proven, cardiovascular risk factors such as diabetes and smoking history, also seem likely to increase risk regardless of cancer type.

Despite extensive investigation, the mechanism responsible for the cardiotoxic effect of anthracyclines remains unknown (Gianni et al., 2008). It is clear that anthracyclines damage the myocyte, yet the timing of the cardiotoxic effect is variable (Bird & Swain, 2008; Gianni et al., 2008). Cardiotoxicity may occur early (within weeks), late (months later) or very late (years) after uncomplicated treatment (Shan et al., 1996). At present, anthracycline induced cardiotoxicity is particularly relevant given the growing number of long-term survivors of childhood cancer (particularly leukemia), and given the variable temporal presentation of cardiotoxicity.

More recently it has been found that a new immunotherapeutic agent, trastuzumab (Herceptin), used to treat breast cancer patients with overexpression of human epidermal growth factor receptor 2 (HER-2)/neu, also has cardiotoxic potential (Piccart-Gebhart et al., 2005; Slamon et al., 2001). As with anthracyclines, the precise mechanism for trastuzumab induced cardiotoxicity remains unclear. Anthracycline exposure is clearly important – cardiotoxicity is worse if trastuzumab is administered in parallel with, rather than following anthracyclines (Rayson et al., 2008). However, the classic ultrastructural changes in myocytes with anthracycline toxicity is not seen in trastuzumab toxicity (Hare et al., 2009), thereby implying a different pathophysiological process. In this regard, it should be noted that HER2/neu receptors play an important role in promoting cell survival during induced myocardial stress (Rayson et al., 2008).

While formal estimates of the incidence of cardiotoxicity consequent to anthracyclines and trastuzumab are variable, its occurrence is of practical importance (Gianni et al., 2008). Given the improved survival of treated patients, it is likely that morbidity and mortality related to anthracycline cardiotoxicity will rise based on the following: 1) significant injury can occur even with (therapeutic) low-dose anthracycline treatment; 2) the techniques currently employed to monitor toxicity are insensitive to subclinical cardiac damage; 3) effective cardio-protection is not systematically administered and 4) ventricular impairment may become evident years after treatment (Shan et al., 1996).

3A.3 CLASSIFYING CARDIOTOXICITY

The mechanisms of cardiotoxicity associated with the use of anthracyclines and trastuzumab are clearly different. The toxic effect induced by anthracyclines is dose related and is not reversible, while that induced by trastuzumab is not dose related and regarded as being largely reversible (Hayes & Picard, 2006). In light of this, it has been proposed that any resultant cardiac dysfunction be classified as follows: dysfunction induced by anthracyclines is type 1 chemotherapy related cardiac dysfunction (CRCD); and the dysfunction induced by trastuzumab is type 2 CRCD (Ewer & Lippman, 2005).

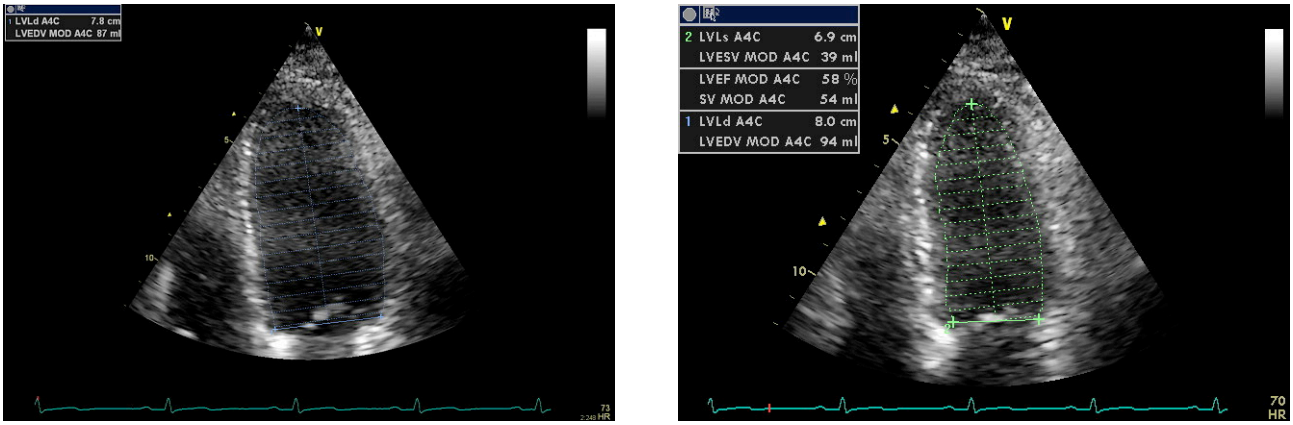
However, having reviewed the findings of 5 major adjuvant trastuzumab trials, Telli et al call into question the theory of reversibility (Telli, Hunt, Carlson, & Guardino, 2007). They agree that improvements in LV function, indicated by improved LVEF may occur in the short-term after withholding treatment. Yet they regard the long-term follow-up in these subjects to be insufficient to determine true reversibility over the longer term. Furthermore, they also note that most of the trastuzumab trials focus largely on reporting severe symptomatic heart failure, thereby neglecting asymptomatic declines in LVEF. This concern seems prudent given that mild asymptomatic LV dysfunction increases the risk of congestive heart failure (CHF) by five times (Wang et al., 2003).

Recognizing distinct cases of type 1 CRCD (dysfunction induced by anthracyclines) and type 2 CRCD (dysfunction induced by trastuzumab when used without an anthracycline) may allow cancer therapy to be based on more refined cardiac risk assessment. However, as most breast cancer regimens for HER2/neu positive patients involve treatment with both agents; this system of classification may have limited practical clinical application.

Nevertheless, in time classifying cardiotoxicity may well prove to be clinically valuable. For now, given that trastuzumab therapy is recent and long term follow up is limited, it appears reasonable to reserve judgment regarding the long term reversibility of trastuzumab. Thus, the early detection and monitoring of cardiac dysfunction remains essential.

3A.4 ECHOCARDIOGRAPHY: CURRENT STANDARDS AND LIMITATIONS

The close monitoring of cardiac function is an important part of managing patients receiving these agents. At present, resting left ventricular ejection fraction (LVEF) by 2-dimensional (2D) echocardiography is the key parameter used to identify and monitor cardiotoxicity (Jurcut et al., 2008a) (see figure 3A.1). LVEF has been validated by comparison with a variety of reference standards, and clear guidelines regarding its acquisition and calculation are published (Lang et al., 2005). Moreover, LVEF provides a single numerical value making comparisons with previous results easy.



A) B)

Figure 3A.1 LVEF measurement involves tracing the LV endocardial border in two orthogonal planes, at end diastole, A) (LVEDV) and end systole, B) (LVESV) enabling calculation of the percentage of volume ejected as: LVEF = (LVEDV – LVESV) / LVEDV. In the example shown, the endocardial border has been traced at end systole in the apical 4 chamber view.

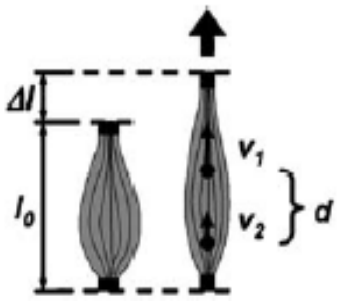
However, despite its established diagnostic and prognostic value, LVEF has numerous limitations. LVEF relies on simplified assumptions about cardiac geometry, hence if the geometry is atypical or abnormal, the measurement is open to error (McGowan & Cleland, 2003). Additionally, LVEF is dependent upon the 2D image quality and on identification of the 'true' LV apex (Malm, Frigstad, Sagberg, Larsson, & Skjaerpe, 2004; Otterstad, 2002). LVEF is a measure of global function and is therefore unable to detect subtle regional alterations in myocardial mechanics, and it is

influenced by variable preload and afterload conditions (Marwick, 2006). Furthermore, LVEF measurement lacks reproducibility; the coefficient of variation being ~ 15% for interobserver variability with test retest variation of ~ 12% (Otterstad, 2002). While a reduced LVEF increases mortality, mild to moderate reductions are not always associated with symptoms (Gianni et al., 2008). With specific regard to anthracycline induced cardiotoxicity, once a clear reduction in LVEF can be demonstrated, functional deterioration proceeds rapidly (Jurcut et al., 2008).

More recently it has been established that heart failure can result from abnormalities of diastolic function, where LVEF is relatively preserved (Zile, 2002). That is, heart failure results from abnormalities that limit cardiac filling and ejection; LVEF may be preserved, while clear signs and symptoms of heart failure are manifest (Gottdiener et al., 2002; Hunt et al., 2009). In this light, the findings of a small prospective study of 26 participants are of considerable interest. Stoddard et al. showed that prolonged Doppler-derived isovolumetric relaxation time (an indicator of diastolic function) preceded and reliably predicted anthracycline-induced systolic dysfunction (as measured by LVEF) (Stoddard et al., 1992). However, larger studies and the criteria for monitoring early changes based on measures of diastolic function in this patient group are lacking and necessary.

3A.5 ECHOCARDIOGRAPHY: NEWER DEVELOPMENTS

The recent development of semi-automated, myocardial strain imaging, provides a more sensitive and reproducible measurement of left ventricular (LV) function. Strain imaging is a measure of cardiac muscle deformation that is expressed as 1) Strain: the percentage of change from the original dimension, and 2) Strain rate: the strain per unit of time (Jurcut et al., 2008b; Yu, Sanderson, Marwick, & Oh, 2007) (see figure 3A.2). Normal ranges for strain imaging have been established, with moderately superior reproducibility values than LVEF (the reproducibility values for strain measurement are reported to vary from 5.5% to 9.5% (Marwick et al., 2009; Sun et al., 2004)).

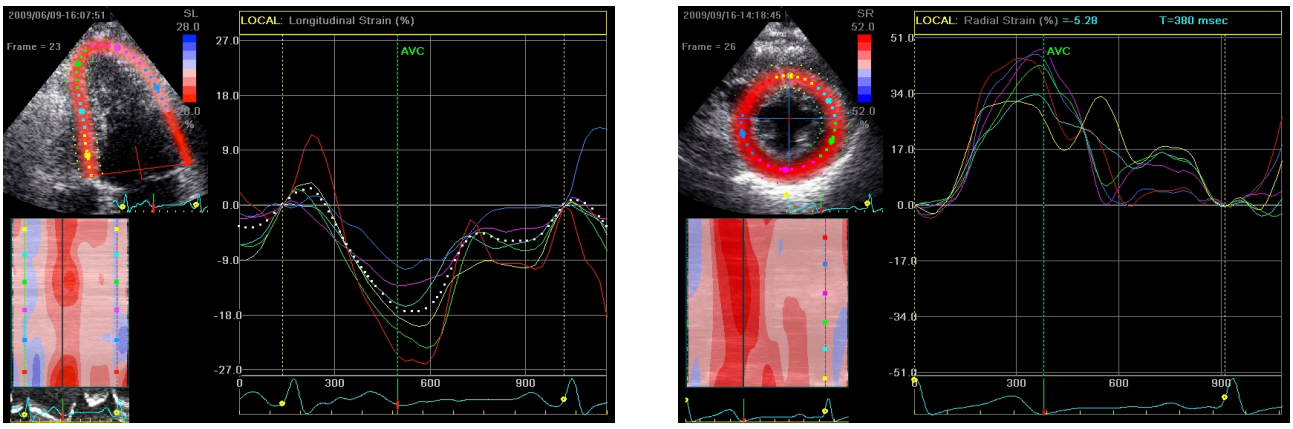


$$Strain = \frac{\Delta l}{l_0}$$

$$Strain\ Rate = \frac{V_1 - V_2}{d}$$

Figure 3A.2 Strain (%): defined as percentage change from the original length. Strain rate (s^{-1}): the instantaneous velocity difference at two points (V_1 and V_2) divided by the distance (d) between them. (Adapted from Jurcut et al. 2008b).

Importantly, strain imaging enables both regional and global assessment of cardiac function. The former frequently precedes global dysfunction, and as discussed earlier, is not captured by the LVEF. Unlike measurement of LVEF, strain does not rely on assumptions about cardiac geometry, it can be made in instances where 2D image quality is compromised, and strain imaging also enables evaluation throughout the cardiac cycle, that is it measures systolic and diastolic function (Ganame et al., 2007) (see figure 3A.3). A recently published work suggests global 2D strain and strain rate to be superior predictors of impaired LV filling than the E/E' ratio (the current standard measure), highlighting the likely value of strain imaging in the assessment of diastolic function (Dokainish, Sengupta, Pillai, Bobek, & Lakkis, 2008).



A) B)

Figure 3A.3 2D speckle tracking, (A) longitudinal and (B) radial strain (%) measurement: The colored lines represent measurements of regional myocardial deformation (the dotted line on the longitudinal strain image represents their 'global' average (all taken from the 2D image at the top left)). ECG at the bottom shows measurements can be made throughout the cardiac cycle.

It has been demonstrated in several disease states, that strain imaging can detect subclinical dysfunction before a noticeable decrease in LVEF (Serri et al., 2006). What is not clear is the longer term clinical relevance of a reduced strain measure, when LVEF is unchanged (Eidem, 2008). In the case of anthracyclines and trastuzumab (as these agents are clearly potentially cardiotoxic) it is likely that strain measurement will be more sensitive to subtle damage to the myocardial ultrastructure, which would otherwise only be confirmed histologically.

Currently, strain measurement is not always a feature of standard ultrasound equipment and strain analysis algorithms differ among manufacturers, making comparison between measurements on different ultrasound systems difficult. Strain imaging requires image acquisition at high frame-rates and careful attention to gain settings in order to minimize noise interference (Leung & Ng, 2010). Most strain measurements require off-line analysis, are time consuming and involve additional training and expertise. Yet, strain imaging itself continues to be refined; it can be derived from tissue Doppler measurements (figure 3A.4), and now from 2D images. The latter requires lower frame rates (40 – 70 frames per second), is relatively angle independent and appears to be more reproducible (Argyle & Ray, 2009). Additionally, 2D strain can evaluate longitudinal, radial and circumferential strain (3 planes) as opposed to Doppler derived strain that largely quantifies longitudinal strain – 2D strain therefore provides a comprehensive measure of LV function.

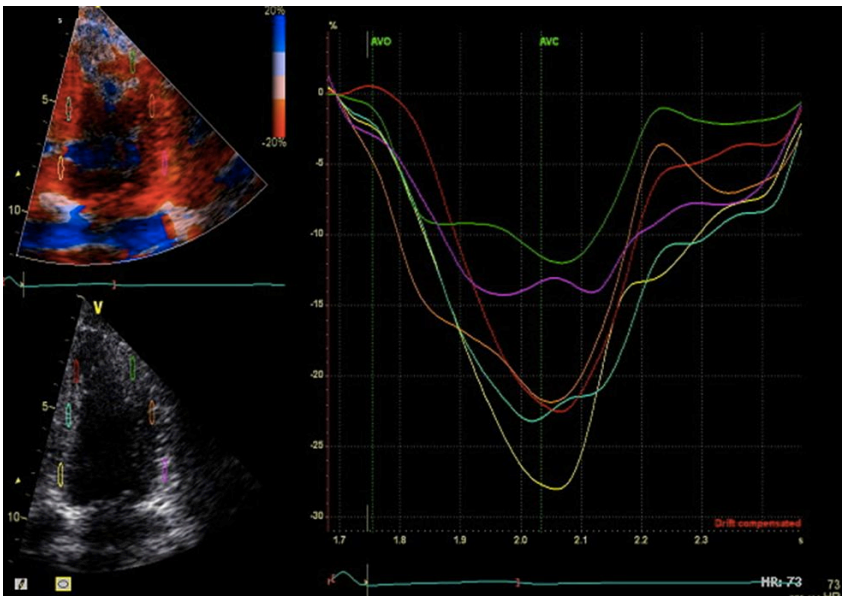


Figure 3A.4 Tissue Doppler imaging longitudinal strain (%) measurement: The colored lines represent measurements of regional myocardial deformation. ECG at the bottom shows measurements can be made throughout the cardiac cycle.

As myocardial strain imaging is still in its infancy, there is limited data regarding strain assessment in the setting of cancer therapy. However, promising results from early work indicate that strain imaging is capable of detecting sub-clinical LV dysfunction, and warrant further investigation. In a study of 56 asymptomatic paediatric patients 5 years post anthracycline treatment, significant reductions in regional Doppler-derived strain and strain rate measurements (especially in the longitudinal direction) were observed while LVEF remained normal (Ganame et al., 2007). Reductions in myocardial function as measured by Doppler and 2D derived strain rate, were observed in 35 breast cancer patients following treatment with trastuzumab while LVEF measurements remained unchanged, with the 2D technique proving more sensitive to acute changes (Hare et al., 2009). In a study of the efficacy of modified anthracyclines with 16 participants, significant reductions were observed in strain and strain rate after 6 cycles of the modified (pegylated) anthracycline, without a significant reduction in LVEF (Jurcut et al., 2008).

As mentioned above, recently published work reveals global 2D strain and strain rate to be superior predictors of LV filling than the current standard measure (Dokainish et al., 2008). Since further research into the diastolic function of patients receiving anthracyclines is warranted, the findings by Tassan-Mangina et al. serve as an important precursor. In a study using both conventional parameters and pulsed tissue Doppler velocity imaging to evaluate 20 adult participants (Tassan-Mangina et al., 2006), early changes in LV diastolic function (that progressed with follow up) occurred prior to changes in LV systolic function.

Moreover, the importance of long term follow up of patients following anthracycline treatment with imaging techniques that are sensitive to early changes, is indicated by another observation from the above mentioned research; 3 years after completion of anthracycline chemotherapy 25% of patients had a significant reduction in LVEF even though LVEF was unchanged 3 months post chemotherapy. Other significant findings include observations from work in rodent models. Abnormal Doppler derived strain indices predicted future cardiac dysfunction and death, when conventional measures (LVEF) remained normal (Neilan et al., 2006). Further, strain and strain rate imaging proved more sensitive to changes in systolic function than standard measurements in a doxorubicin-induced cardiomyopathy model (Piegari et al., 2008).

The value of myocardial strain imaging in clinical practice is yet to be fully realized. Clearly, in the research setting it already provides a more refined tool to evaluate regional and global cardiac function. At present, there is no strain or strain rate value that provides a cut-off beyond which clinically manifest symptoms are more likely to occur, nor clear evidence showing

strain or strain rate to be a superior method. Abnormal values may be difficult to define, especially in the setting of multiple coexisting risk factors. However, strain will likely improve detection of within patient change by providing a more sensitive measure of the effects of cardiotoxic agents. This may improve decision making regarding the cessation of cancer treatment if needed and to the addition of potential supportive therapies (including angiotensin converting enzyme inhibitors and beta blockers) to improve cardiac function.

One of the considerable challenges of treatment with anthracyclines relates to the fact that there is no clear 'curative dose' response relationship, nor a 'cardiotoxicity dose' response relationship. Any method of achieving the 'curative dose', whether by administration of a cardioprotective agent or by modification of the administered chemotherapy agent, will require monitoring of changes, using methods such as strain imaging that are sensitive to subtle early changes.

3A.6 CONCLUSION

The single greatest risk for the development of significant heart failure related to anthracyclines is the early development of cardiotoxicity. By the time cardiotoxicity is detected by a significant reduction in LVEF, functional deterioration often proceeds quickly. Myocardial strain imaging, with its ability to measure systolic and diastolic function, may permit early detection of subclinical regional or global cardiotoxicity. However, to date there is no prognostic data that demonstrates that the early detection of cardiotoxicity by strain will alter clinical prognosis. Such data would facilitate modification of chemotherapy together with the introduction of therapy to minimize the impact of cardiotoxicity.

The development of cardiotoxicity is a lifelong concern following anthracycline treatment as it occurs at varying times after therapy. Even if a more effective anticancer agent without cardiotoxicity is developed to replace anthracyclines, the number of patients who have received anthracyclines to date is considerable and this cohort will require continued and accurate long term monitoring. Likewise, sensitive measures of cardiac function are needed for the longer-term follow up of those treated with trastuzumab.

The necessary long-term cardiac monitoring of cancer patients treated with anthracyclines and/or trastuzumab must be safe, reproducible and cost effective. Echocardiographic myocardial strain imaging is a promising clinical modality for early detection of cardiotoxicity and for long-

term surveillance of cancer patients. Further research is warranted in order to determine its role in this important clinical setting.

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CHAPTER 3 (PART B)

EXTENDED LITERATURE REVIEW

Preamble

The review article entitled 'The potential role of echocardiographic strain imaging for evaluating cardiotoxicity due to cancer therapy' presented in part A was reduced by editorial constraints. Both the length of the review and the number of references included were limited, therefore an additional section (part B) is presented below. Moreover, as 3 years have elapsed since the publication of the original review Part B provides greater detail and additional recent information that was unavailable when part A was published: specific reference is given to anthracycline chemotherapy and the development of strain imaging and its potential benefits for evaluating cardiotoxicity. While the arrangement of this section is purposely similar to part A, there is very little overlap of content in the two chapters, thus part B completes the literature review.

3B.1 ANTHRACYCLINE AGENTS AND CARDIOTOXICITY

3B.1.1 EXTRACTION AND EFFICACY

Anthracycline antibiotics are natural compounds originally derived from Streptomyces bacteria (Arcamone, et al., 1974). Their potent anti-tumor properties were recognized soon after their discovery, and anthracyclines have been used extensively ever since (Arcamone, et al., 1974). The administration of these agents has been central in the improved life expectancy for victims of numerous cancers, including breast cancer ((EBCTCG), 2005; Arcamone et al., 1997). Four decades after their development, anthracycline agents remain the cornerstone of breast cancer treatment; the two anthracyclines most widely used in this setting are doxorubicin and epirubicin, which as shown in figure 3B.1, are structurally similar (Burstein et al., 2012; Gianni et al., 2008).

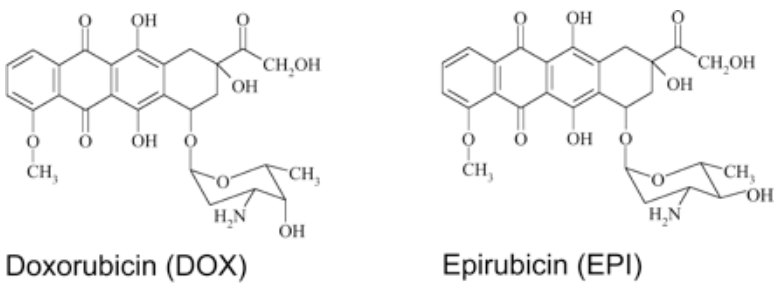


Figure 3B.1 Illustration of the molecular structure of Doxorubicin and Epirubicin anthracycline antibiotics. (Adapted from Xu, Pearson & Richardson, 2005).

While anthracyclines afford clear benefits in the treatment of cancer, the way in which they achieve their anti-tumor actions is not completely understood. What is known, is that anthracyclines enter neoplastic cells by passive diffusion, where they bind with proteasomes in the cytoplasm, and as a drug-proteasome complex are transported into the cells nucleus (Kiyomiya, Matsuo, & Kurebe, 2001; Skovsgaard & Nissen, 1982). Their binding with proteasomes is one method of inducing apoptosis in neoplastic cells, as it inhibits the normal pro-survival protein degrading activity of protease (Kiyomiya et al., 2001). Once inside the nucleus, anthracyclines dissociate from proteasomes and bind to deoxyribonucleic acid (DNA) (for which they have a high affinity) (Minotti, Menna, Salvatorelli, Cairo, & Gianni, 2004); this enables their varied activity on neoplastic cell DNA (including the direct inhibition of DNA transcription) (Minotti et al., 2004; Xu,

Persson, & Richardson, 2005). Other cytotoxic effects caused by anthracyclines include lipid peroxidation and the generation of reactive oxygen species (free radicals) (Gewirtz, 1999).

3B.1.2 CARDIOTOXICITY

Unfortunately, the efficacy of anthracyclines is undermined by their detrimental effects on nonmalignant cells - cardiotoxicity (injury to cardiac myocytes that leads to structural changes such as vacuolization in the myocytes and weakens contractility) being particularly detrimental (Moriyama, Kemi, Okumura, Yoshihara, & Horie, 2010; Singal, Li, Kumar, Danelisen, & Iliskovic, 2000) (see figure 3B.2). Interestingly though, the cardiotoxic mechanisms of anthracyclines differ from their therapeutic actions (Sawyer, Peng, Chen, Pentassuglia, & Lim, 2010). The primary means of anthracycline-induced cardiotoxicity is free radical formation that leads to DNA damage (Sawyer et al., 2010; Shaikh & Shih, 2012). Other means include the induction of respiratory defects in myocardial mitochondria and transcriptional changes in myocardial adenosine triphosphate production. Anthracyclines also disrupt the function of the basic mechanical unit of myocytes, the sarcomere, by degrading the essential structural protein Titin (see figure 3B.4) (Chen, Peng, Pentassuglia, Lim, & Sawyer, 2007; Sawyer et al., 2010; Yeh & Bickford, 2009).

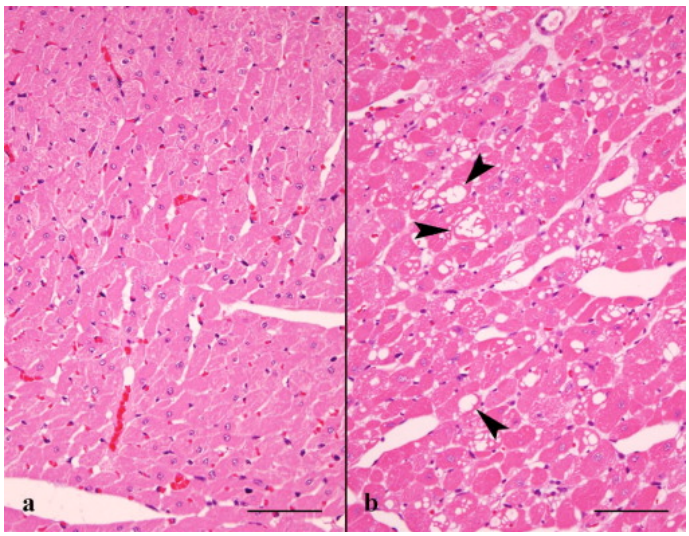


Figure 3B.2 Histology sections of murine cardiac myocytes: a) a control animal, b) an animal treated with doxorubicin - the arrow heads point to areas of vacuolization in the myocytes, indicative of doxorubicin-induced injury. (Adapted from Moriyama, 2010).

The most important factor related to anthracycline-induced cardiotoxicity is the cumulative anthracycline dose (Swain, Whaley, & Ewer, 2003). Dose-limiting strategies reduce cardiotoxicity, however patients still develop cardiac dysfunction, and there is considerable variation in the

reported frequency of anthracycline-induced cardiotoxicity. In adults, late onset clinical heart failure occurs in up to 5% of patients more than 1 year post therapy (Shaikh & Shih, 2012). In pediatrics, 75% of cancer survivors develop at least one chronic physical health condition 30 years post treatment - some of which are a direct result of the cardiotoxic effects of anthracycline chemotherapy (Shaikh & Shih, 2012).

Management of cardiotoxicity is further confounded by its temporal variability. Acute cardiotoxicity occurs in less than 1% of patients immediately after infusion (Shaikh & Shih, 2012). Early-onset chronic cardiotoxicity occurs in 1.6 - 2.1% of patients within 1 year of therapy (with a peak incidence at 3 months post treatment). Late-onset chronic cardiotoxicity occurs in ~ 5% of patients more than 1 year post therapy (Shaikh & Shih, 2012).

3B.1.3 CURRENT MONITORING STANDARDS AND LIMITATIONS

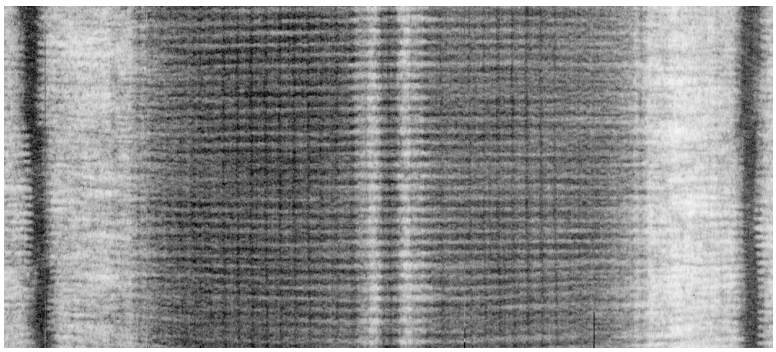
The beneficial and detrimental effects of anthracyclines are well recognized. However, clear guidelines for the detection and management of cardiac dysfunction after anthracycline chemotherapy are lacking. Appropriately timed noninvasive evaluation of cardiac function is considered to be essential (Wells & Lenihan, 2010), yet the current standard method used in clinical practice, the measurement of LVEF, is limited. LVEF essentially measures global LV volumetric change; for the reasons previously outlined (in chapter 3, part A), and those given below, this does not enable early cardiac dysfunction to be adequately measured. Once LVEF is significantly reduced, restoration of normal LV function is difficult (Cardinale et al, 2010).

3B.2 ECHOCARDIOGRAPHY AND THE EVALUATION OF MYOCARDIAL MOTION

3B.2.1 LEFT VENTRICULAR ANATOMY

Left ventricular (LV) muscular fiber anatomy and function are complex. The mechanical action of the LV is the result of work performed by a network of specialized muscle cells, cardiac myocytes. The myocytes are filled with long parallel myofibrils, which contain protein rich contractile filaments, myosin and actin; the contractile filaments comprise the basic mechanical unit of each myocyte, the sarcomere (Guyton & Hall, 1996; Sherwood, 1993) (see figure 3B.3). Myofibrils are surrounded by a network of sarcoplasmic reticulum (SR); in response to stimuli, calcium is released from the SR and its activating action on the myofilaments is an essential part of the energy (ATP) dependent process of myocardial contraction (Sherwood, 1993). By way of

desmosomes, myocytes adhere to their neighboring cells (and communicate via gap junction), and are supported by fibrous connective tissue (Anderson, Ho, Redmann, Sanchez-Quintana, & Lunkenheimer, 2005; Sherwood, 1993).



Sarcomere

Figure 3B.3 Electron microscope image of a sarcomere, the basic mechanical unit of the myocyte. The protein rich contractile elements, myosin and actin, help to give myocytes their characteristic striated appearance. (Adapted from Luther, 2013).

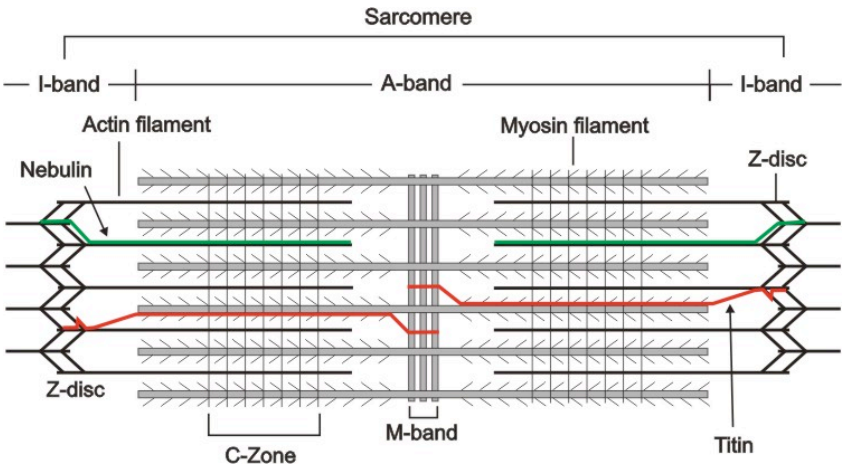


Figure 3B.4 Detailed illustration of the elements that comprise the sarcomere. (Adapted from Luther, 2013).

Overall, the LV is conical in shape (Skinner J, 2000). Its muscle mass has a helical configuration, with 3 functional muscle 'layers' within its wall. The muscle layers do not comprise discrete muscle groups, but are classified according to the predominant orientation of myocytes (Anderson et al., 2005; Sengupta & Narula, 2012) (see figure 3B.5A). While their overall orientation is distinct, for normal contraction the 'layers' operate together in a number of simultaneous events (Armstrong & Ryan, 2010). At the onset of contraction, the myocardium begins to thicken, the

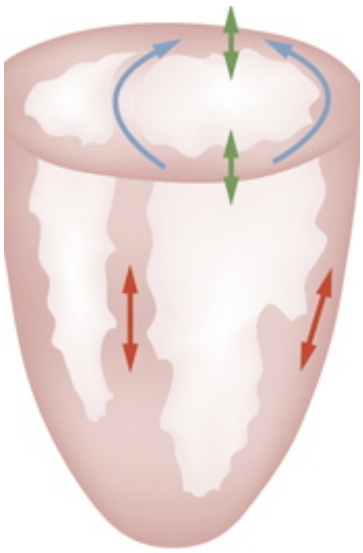
endocardium moves toward the centre of the ventricle, and the LV chamber diameter and length are reduced (Armstrong & Ryan, 2010). Together with this inward motion, clockwise rotation at the base and anti-clockwise rotation at the apex occur to produce a highly efficient 'wringing' motion for the essential movement of blood from the ventricles (Sengupta et al., 2007).

3B.2.2 STRAIN IMAGING

LVEF provides an estimate of the change in LV volume. While three-dimensional echocardiography (3DE) has been shown to be a more accurate method than 2D (Jenkins, Bricknell, Chan, Hanekom, & Marwick, 2007) in the measurement of LVEF, neither method is well suited to evaluating myocardial function. The left ventricle has considerable functional reserve; significant myocardial damage may occur before a reduction in LVEF (Ewer & Lenihan, 2008), and patients may remain asymptomatic until a reduction is observed. Myocardial strain imaging promises to improve evaluation of LV function by echocardiography: in contrast to LVEF, strain enables a direct measurement of myocardial motion (Perk, Tunick, & Kronzon, 2007). LV architecture and function is complex (see figure 3B.5 A); strain imaging enables quantification of segmental and global longitudinal, circumferential, and radial LV myocardial motion (Geyer et al., 2010a; Gorcsan & Tanaka, 2011; Ozkan, Kapadia, Tuzcu, & Marwick, 2011) (see figure 3B.5 B).

A)

Figure 3B.5 A) An image of the complex arrangement of muscle fibers that comprise the left ventricle**.** (Adapted from Gorcsan, 2011).



B)

Figure 3B.5 B) Illustration of the directions of myofiber motion; longitudinal (red arrows), circumferential (blue arrows), radial (green arrows). (Adapted from Ozkan, 2011).

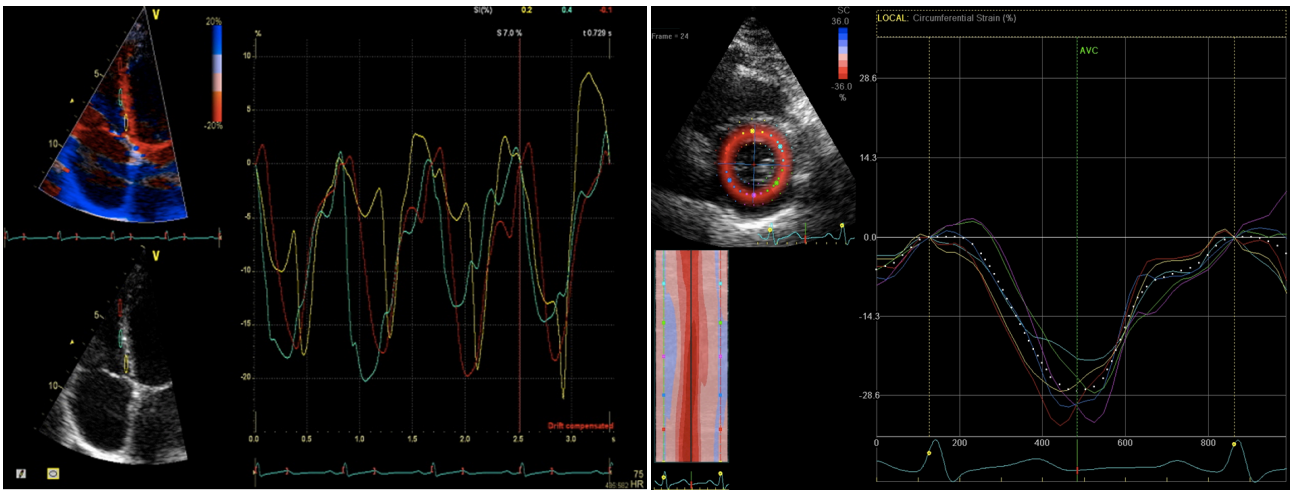
When matter is subjected to a load, it is deformed. Strain is a measure of deformation, which may be elastic (recoverable) or plastic (permanent) (van Vluck, 1982). Quantitative measures of strain are possible - strain is used extensively in engineering for this purpose. For example, the suitability of materials used in the building and automotive industries can be evaluated by testing their deformation (van Vluck, 1982). Strain is a dimensionless index that represents the percentage change in the length of an object in response to stress (Leung & Ng, 2010; Sengupta et al., 2007), and is determined by the following equation (Leung & Ng, 2010):

$$\textbf{\textit{Strain}} = (L - Lo) / Lo$$ *(where: L = length after deformation; Lo = original length)*

Strain is defined as being positive when the distance between the points of measurement increases and negative when this distance decreases. In echocardiography transmural thickening (radial strain) is denoted as positive, and longitudinal shortening and circumferential movement as negative (Leung & Ng, 2010) (see image 3B.5 B). In simple terms, a 10 cm object stretched to 12 cm would have a 20% positive strain (Mor-Avi et al., 2011b).

3B.2.3 TECHNICAL ASPECTS OF STRAIN IMAGING

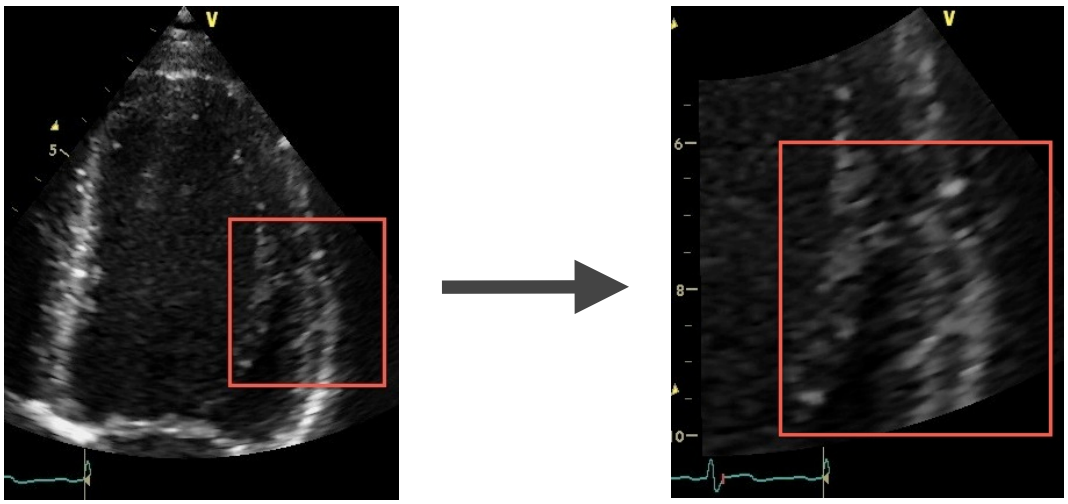
Two echocardiographic methods can be used to measure strain; tissue Doppler imaging (TDI) and two-dimensional speckle tracking (Argyle & Ray, 2009). TDI was the first method for quantifying myocardial strain (Geyer et al., 2010b) (see figure 3B.6 A). By calculating the relative velocity of motion at a location in space as a function of time, TDI derived strain is measured without reference to the initial myocardial length; this means of calculating strain is referred to as ‘spatial’ or ‘Eulerian strain‘ (Geyer et al., 2010b; Leung & Ng, 2010). TDI is almost universally available on ultrasound machines, and can be analyzed on or offline. However, the TDI method has a number of limitations including the need for parallel orientation of the ultrasound beam with the direction of myocardial motion (Geyer et al., 2010b) (see table 3B.1 for pros and cons of the TDI method).



A) B)

Figure 3B.6 A) Longitudinal myocardial strain calculated using the TDI method, B) circumferential myocardial strain (calculated using the two-dimensional speckle tracking method), which displays negative deformation. (For an image of radial strain measurement see figures 3A and 3B in part A of chapter 3).

Two-dimensional (2D) echocardiogram images have a speckled pattern that is the result of reflections from natural acoustic markers (smaller than the ultrasound wavelength) within the myocardium (Leung & Ng, 2010; Mor-Avi et al., 2011b; Sengupta et al., 2007) (see figure 3B.7). By grouping the speckles into 'blocks', they can be tracked frame-by-frame and used to measure myocardial deformation: this second (contemporary) method of measuring strain is referred to as 2D speckle tracking echocardiography (2DSTE) (Armstrong & Ryan, 2010; Koopman et al., 2010; Mor-Avi et al., 2011a) (see figure 3B.8). With 2DSTE, myocardial length is known before, during and after deformation, which enables the calculation of strain using the 'material' or 'Lagrangian' method (Armstrong & Ryan, 2010; Koopman et al., 2010; Mor-Avi et al., 2011a).



A) B)

Figure 3B.7 A) Apical 4 chamber image of the LV with an area of the lateral wall highlighted, and B) zoomed image of the lateral LV demonstrating the speckled grey-scale pattern.

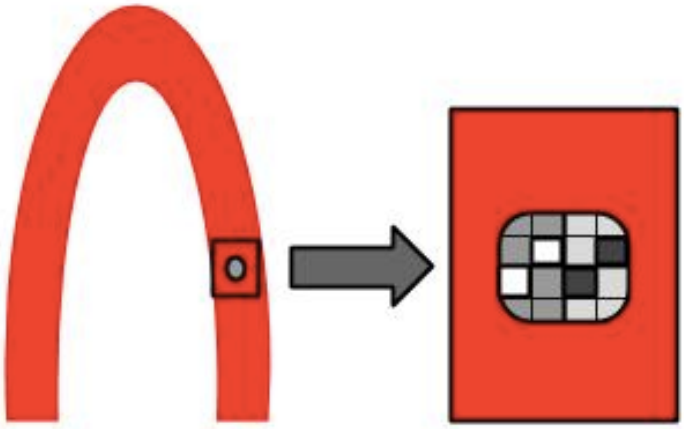


Figure 3B.8 Depiction of the speckle pattern from a region of the lateral LV (as seen from an apical 4 chamber image). In 2DSTE, speckles are arranged into blocks which have a unique pattern of speckles and can be tracked frame-by-frame to measure myocardial deformation.

The spatial resolution of the speckle method for measuring strain is superior to the TDI method (Geyer et al., 2010b; Reisner et al., 2004). 2DSTE allows angle independent quantification of strain and has the additional capacity to calculate global strain (by averaging the results from all segments of the left ventricle) (Argyle & Ray, 2009). Inferior temporal resolution limits the 2DSTE method more than TDI, and the speckle method is less readily available than the TDI method (see table 3B.1 for the pros and cons of the 2DSTE method). 2DSTE is the method used and investigated in this research and is the method referred to (unless specifically stated otherwise) throughout the thesis.

Table 3B.1 Advantages (pros) and disadvantages (cons) of the two echocardiographic methods for measuring myocardial strain

	Tissue Doppler Imaging	2D Speckle Tracking Echocardiography
Pros	- High temporal resolution - Software readily available - On or off-line analysis	- Angle independent - Segmental and global strain analysis - Semi-automated
Cons	- Angle dependency - Sample volume variability - No global strain calculation	- High resolution image dependency - Software less available

3B.2.4 STRAIN RESEARCH METHODOLOGY

Detailed description of the methods used in the research presented in this thesis are given in the methods section of chapters 4, 5, 6 and 7. These include general details (unrelated to strain measurements), as well as those specifically related to the utilization of strain imaging such as the views used for image acquisition, the criteria employed to ensure the appropriate timing of analysis, and the criterion for verify acceptable 2D image tracking quality. Moreover, the number of images analyzed in each view is also specified. (For these specific details, refer to the methods sections of the above mentioned chapters).

One aspect of strain measurement that is not detailed in the methods sections of the published papers presented in chapters 4, 5, 6 and 7, is the myocardial tracking quality assessment 'intrinsic' to the strain imaging software. As this process is determined by the manufacturer (GE healthcare), it was omitted from the published papers. However, it is worthwhile noting here, that strict criteria are applied in imaging software to ensure appropriate tracking of speckles. That is, speckles are given a low tracking quality score or excluded if (Perk et al., 2007):

- they do not return to a baseline position after forward and backward motion,

- adjacent speckles have significantly different velocities,

- strain measurements differ at the beginning and the end of a cardiac cycle

3B.3 STRAIN ANALYSIS FOR THE DETECTION OF CARDIOTOXICITY

Strain imaging offers an opportunity to improve the sensitivity of echocardiography in the detection of LV dysfunction: global longitudinal strain (GLS) has proven to be particularly valuable in this case. A contemporary study aimed at identifying the echocardiographic technique that adds the greatest predictive value to clinical variables, revealed GLS is a superior predictor of outcome to either LVEF or wall motion score index (WMSI) (Stanton, Leano, & Marwick, 2009). The primary reason why GLS is likely the most useful strain measure, is that longitudinal strain is acquired from the apical window, and from the apical window all LV segments can be evaluated (Mor-Avi et al., 2011b).

Myocardial insults such as ischemia and inflammation have been shown to affect the subendocardial, longitudinal fibers prior to other fibers. The optimal function of longitudinal fibers are measured most effectively with longitudinal strain. For example, myocardial longitudinal function (measured by 2D strain) is impaired at rest in most patients with coronary artery disease (CAD) (Choi et al., 2009), can be used to predict the presence of significant CAD (Shimoni et al., 2011), and provides useful information about the extent of myocardial fibrosis in hypertrophic cardiomyopathy (even when LVEF is normal (Saito et al., 2012; Yajima et al., 2012). These reports are evidence for the value of longitudinal strain imaging, albeit in varying clinical scenarios.

For the detection of LV dysfunction in the setting of cancer therapy, reduced longitudinal and radial strain using the TDI technique has been reported in a small study of 16 patients over the age of 65 after treatment with pegylated anthracyclines (Jurcut et al., 2008). Moreover, since the publication of the first review (chapter 3 part A), several studies demonstrating the value of the 2D speckle technique for detecting cardiotoxicity have been published: reduced longitudinal strain and preserved LVEF in long-term cancer survivors treated with anthracyclines and or radiation therapy (RT) has been reported (Tsai et al., 2011).

In the detection of LV dysfunction in the setting of breast cancer therapy, significantly reduced GLS after 3 months of trastuzumab (following anthracyclines), has been reported in patients with a subsequent decline in LVEF of ≥10% to <55% at 6 months (when LVEF at 3 months was normal) (Fallah-Rad et al., 2011). Similarly, significantly reduced GLS after 3 months of trastuzumab (following variable anthracycline exposures) has been reported to predict cardiotoxicity. In this study cardiotoxicity was defined as a reduction in LVEF ≥5% to <55% with symptoms of heart failure or an asymptomatic reduction of the LVEF ≥10% to <55% (as per the

Cardiac Review and Evaluation Committee (CREC) criteria), while LVEF at 3 months was not predictive of toxicity (Sawaya et al., 2011). More recently, an 11% reduction in GLS (from the baseline value) 6 months into trastuzumab, has been proposed as the optimal cut-off value for predicting trastuzumab-induced cardiotoxicity (with toxicity classified according to the CREC criteria) (Negishi et al., 2013).

3B.4 A MULTI-MODALITY APPROACH FOR PREDICTING CARDIOTOXICITY

Modern medicine utilizes a multi-modality approach for detecting and managing disease. Echocardiographic strain imaging shows great potential value in the detection and management of cardiotoxicity: its value in this regard would be enhanced when used in combination with other monitoring techniques (Wells & Lenihan, 2010). For example, there is growing evidence for the usefulness of blood serum biomarkers in the early identification of anthracycline-related cardiotoxicity (Dolci, Dominici, Cardinale, Sandri, & Panteghini, 2008). Early and persistent elevation of cardiac troponin (the biomarker of choice for identifying myocardial injury) has been shown to identify patients who are more likely to develop symptomatic heart failure and benefit from supportive therapies (Cardinale & Sandri, 2010). The importance of the early identification of anthracycline induced LV dysfunction has recently been underscored by the results of a biomarker study, which demonstrated that successful response to supportive therapies (angiotensin-converting-enzyme inhibitors and beta blockers) is significantly reduced if therapy is not promptly introduced (Cardinale et al., 2010).

As described in part A of the literature review, anthracycline chemotherapy is not the only potential source of cardiac damage in breast cancer patients. Approximately 25% of breast cancer patients over express the human epidermal growth receptor 2 (HER2/neu) protein - for these patients the introduction of trastuzumab has brought considerably improved survival (Piccart-Gebhart et al., 2005; Slamon et al., 2001). However, trastuzumab also has a 'dark side': up to 14% of patients will require a delay to, or the suspension of their treatment due to significant LV disfunction (Hayes & Picard, 2006; Telli, Hunt, Carlson, & Guardino, 2007). A contemporary study (reported following publication of the initial review) of HER2/neu positive patients indicates that peak systolic longitudinal strain and cardiac troponin measured after anthracyclines and prior to trastuzumab are helpful in predicting patients who develop cardiotoxicity (as defined by the CREC criteria) during trastuzumab therapy (Sawaya et al., 2012). In this report, no patient with a peak systolic strain value <-19% after anthracyclines developed trastuzumab-related cardiotoxicity.

In addition to anthracyclines and trastuzumab, many breast cancer patients also receive breast or chest wall radiotherapy (RT). In this case, the heart is further subjected to potentially toxic therapy (Patt et al., 2005). While important advances in RT ensure that modern techniques deliver well directed therapy with minimal doses to the heart (Doyle et al., 2007; Patt et al., 2005), contemporary reports recognise that with RT there is increased risk of heart disease, and that RT augments the cardiotoxic effect of anthracycline chemotherapy (Darby et al., 2013; Tsai et al., 2011). Prospective analysis of breast cancer patients exposed to anthracyclines (with or without trastuzumab) and RT with myocardial strain imaging and biomarker analysis are needed.

3B.5 CONCLUSION

Anthracyclines remain an essential part of treatment regimens for numerous cancers. Their effective anti-tumor properties are a primary reason for improvements in life-expectancy. However, significant side effects, especially cardiotoxicity, continue to undermine their administration. Myocardial strain imaging is contemporary technology that uses the acoustic properties inherent to cardiac myocardium to derive measurements of deformation. Strain imaging technology may improve monitoring of cardiotoxicity, and aid in the development of imaging protocols aimed at further improving outcomes. At the start of this research this hypothesis had been largely untested: the remaining chapters of the thesis detail the results of research aimed at examining this hypothesis.

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CHAPTER 4

TWO-DIMENSIONAL MYOCARDIAL STRAIN IMAGING DETECTS CHANGES IN LEFT VENTRICULAR SYSTOLIC FUNCTION IMMEDIATELY AFTER ANTHRACYCLINE CHEMOTHERAPY

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Preamble

The preceding chapter detailed the potential role of echocardiographic strain imaging for evaluating cardiotoxicity due to cancer therapy, with particular reference to its utility when anthracycline agents are used in the management of breast cancer. While anthracyclines are used in the management of other malignancies, they have been the cornerstone of breast cancer treatment for decades. Anthracycline based regimens used in breast cancer management vary little between patients, and the majority of breast cancers are detected early, which makes these patients an ideal group to study. This chapter presents the published findings of the analysis of left ventricular (LV) systolic function with strain imaging, made immediately after anthracycline chemotherapy, in the first fifty-two consecutive breast cancer patients.

ABSTRACT

AIMS

The efficacy of anthracyclines is undermined by potentially life threatening cardiotoxicity. Cardiotoxicity is dependent upon several factors, and the timing to its development is variable. Moreover, as adjuvant therapy with trastuzumab often follows, close monitoring of cardiac function in those treated with anthracyclines is mandatory. LVEF by echocardiography is currently used for monitoring cardiotoxicity, however LVEF has numerous limitations. Two-dimensional strain imaging may provide a more sensitive measure of altered LV systolic function, so the aim of the present study was to compare LVEF and LV systolic strain before and after anthracyclines.

METHODS AND RESULTS

Fifty-two women with histologically confirmed breast cancer were prospectively studied. Echocardiographic LVEF (by Simpson's method), global and regional peak longitudinal, radial and circumferential 2D systolic strain was measured 1 week before and 1 week after chemotherapy.

Global and regional longitudinal LV systolic strain was significantly reduced after treatment; global longitudinal strain decreased from -17.7% to -16.3% ($p < 0.01$) with 48% of global measurements reduced by >10%. Global and regional radial LV systolic strain after treatment was also significantly reduced; global radial strain dropped from 40.5% to 34.5% ($p < 0.01$) with 59% of global measurements reduced by >10%. In contrast, no reduction in LVEF >10% after chemotherapy was observed.

CONCLUSION

Reduced LV systolic strain immediately after anthracycline treatment may indicate early impairment of myocardial function before detectable change in LVEF.

4.1 INTRODUCTION

Four decades following their discovery, anthracyclines remain among the most widely prescribed anticancer agents; however their efficacy is undermined by potentially life threatening cardiotoxicity (Lefrak, Pitha, Rosenheim, & Gottlieb, 1973; Shan, Lincoff, & Young, 1996; Singal, Li, Kumar, Danelisen, & Iliskovic, 2000). As cardiotoxicity is dependent upon a number of treatment and patient related factors and the timing of its development is variable (Bird & Swain, 2008; Gianni et al., 2008), close monitoring of cardiac function before, during, and after anthracycline treatment is mandatory (Bird & Swain, 2008; Singal & Iliskovic, 1998).

Currently, resting left ventricular ejection fraction (LVEF) by two-dimensional (2D) echocardiography is the key parameter used to identify and monitor anthracycline-induced cardiotoxicity. LVEF, however, has numerous technical limitations and is a measure of global function, unable to detect subtle regional changes in myocardial mechanics (Jurcut et al., 2008; Malm, Frigstad, Sagberg, Larsson, & Skjaerpe, 2004; McGowan & Cleland, 2003; Otterstad, 2002). The recently developed myocardial strain imaging may provide a more sensitive and reproducible measurement of altered LV systolic function.

The aim of the present study was to compare LVEF and LV systolic strain measured with two-dimensional (2D) strain imaging before and immediately after anthracycline chemotherapy. We hypothesized that global LV systolic strain measures would prove more sensitive than LVEF in detecting early anthracycline-induced systolic dysfunction, and would detect subclinical regional LV systolic dysfunction prior to overt changes in global function.

4.2 METHODS

Ethics approval for the study was granted by the Sydney West Area Health Service Research Ethics Committee and the University of Sydney, and written informed consent was obtained from all participants. Fifty-two consecutive anthracycline naïve women with histologically confirmed breast cancer were prospectively recruited prior to any thoracic radiotherapy. All participants were recruited at initial review at Westmead Hospital, although subsequent cancer treatment was administered at one of four affiliated sites (depending on proximity to the patient's residence). A detailed record of the administered cumulative anthracycline dose was documented.

The clinical history, physical examination and echocardiogram were used to establish eligibility. Exclusion criteria included LVEF before chemotherapy of <50%, rhythms other than sinus, more than mild valvular stenosis or regurgitation, prosthetic valve or pacemaker. A detailed cardiac history was obtained at recruitment including clinical risk factors for heart disease (hypertension, diabetes, cholesterol, smoking history, and diabetes). Height was measured at baseline, while weight and blood pressure were measured at the time of each echocardiogram. Patients were also evaluated for any cardiac symptoms especially those of cardiac failure.

The initial echocardiogram was performed prior to the commencement of chemotherapy (ideally 1 week before). The follow-up echocardiogram was performed 1 week following completion of chemotherapy (i.e. 12 or 18 weeks after the first study, depending on whether the patient had 4 or 6 cycles of chemotherapy respectively) and always before commencement of trastuzumab or thoracic radiotherapy. All echocardiograms were performed at a single site by either of two experienced sonographers using a Vivid 7 digital ultrasound system (GE medical systems, Norway) with a 2.5MHz variable frequency transducer.

A comprehensive transthoracic echocardiogram was performed with patients in the left lateral position. Images were obtained from the parasternal, apical and subcostal views. LVEF was measured by Simpson's method according to the recommendations of the European Association of Echocardiography (Lang et al., 2006), and measured at the time of acquisition without reference to previous measurements. Clinically significant reductions in LVEF were defined as ≥ 10% from before chemotherapy, or an absolute LVEF < 50% after chemotherapy.

Depth adjusted 2D images of the left ventricle in the parasternal short axis at the level of the papillary muscles, and in the apical 4, 2- and long axis views were acquired and stored for off-

line measurement of 2D speckle tracking myocardial strain (with EchoPac PC versions 6.0, GE Healthcare, UK) (see figure 4.1). Frame rates were optimized at the time of acquisition to between 50 and 70fps. To ensure reproducibility and accuracy, systole was defined as the interval from aortic valve opening to closure, measured with pulsed wave Doppler sampling of blood flow in LV outflow tract.

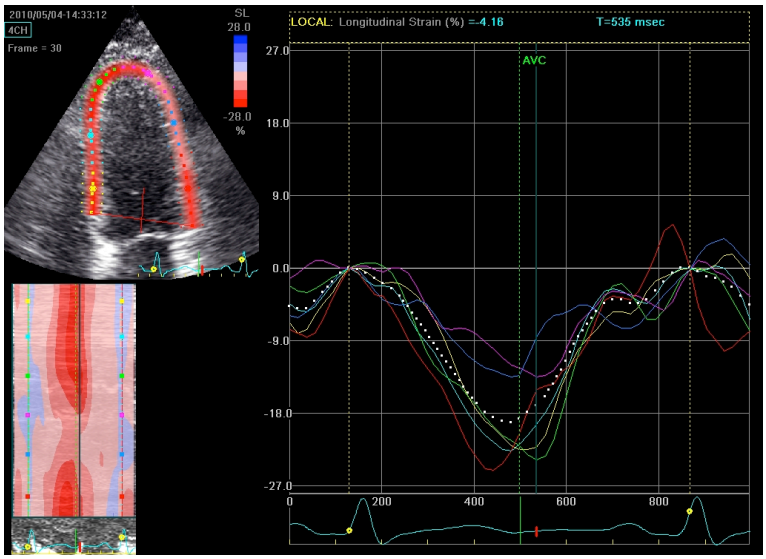
The LV regions of interest for strain analysis were manually selected by marking the endocardial border at end systole in the appropriate imaging plane. Traces from each segment were inspected to confirm that peak systolic strain was measured prior to aortic valve closure and the tracking quality of all images was identified before analysis using the software scoring table on the off-line measuring package. Tracking quality was overridden in segments with no more than 2 rejected regions where the observer deemed tracking quality to be clearly acceptable. Images with persistently unacceptable tracking quality (more than 2 segments) were excluded from the final analysis.

Strain measurements were made from 3 consecutive cardiac cycles and then averaged in order to obtain peak systolic regional strain and strain rate in the longitudinal, radial and circumferential planes (see figure 4.1). Global peak systolic strain and strain rate was calculated by averaging the 6 regional values in the apical 4 chamber and parasternal short axis view at the mid papillary level to measure longitudinal, radial and circumferential strain respectively. Additionally, in a subgroup of 19 participants with optimal image quality, longitudinal strain was measured from the apical 2 and long axis views and global (apical triplane) longitudinal strain was calculated as an average of 18 segments.

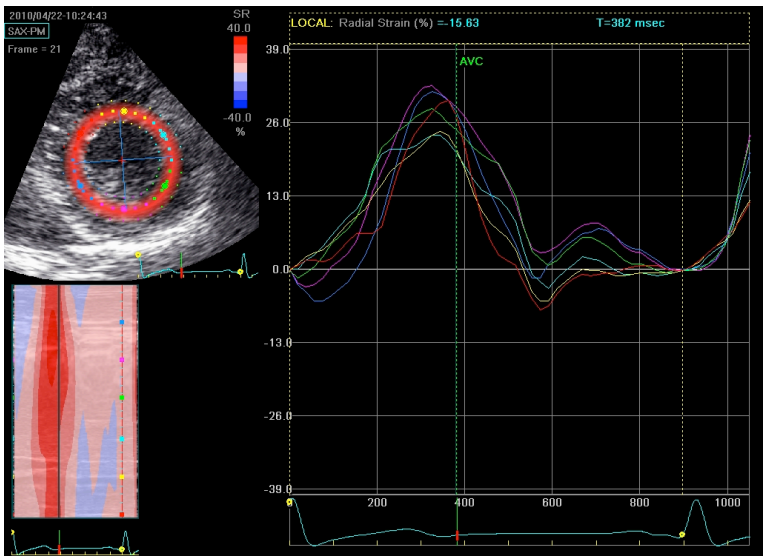
Ten participants in whom longitudinal, radial and circumferential strain measurements were all possible were randomly selected in order to calculate interobserver and intraobserver variability, with differences reported as mean difference ± standard deviation (SD). LV systolic strain and LVEF measurements were repeated by a different observer (blinded to previous results) to measure interobserver variability. Intraobserver variability for LV systolic strain and LVEF were assessed by the same observer on a different occasion from the same digital data, using an offline system. Differences are reported as mean error ± SD of 10 measurements.

All values were expressed as a mean ± SD unless otherwise stated. The effect of anthracycline therapy was examined with a repeated measures analysis of variance (ANOVA). Paired t tests were used to compare LV segmental strain parameters before and after

chemotherapy. Linear regression analysis and Pearson correlations were performed to examine the relationship of LV systolic strain parameters to cumulative anthracycline dose and age. Chi square analysis was used to assess the effect of baseline clinical risk factors on altered LV systolic function at follow-up, while univariate analysis was performed to evaluate the effect of alteration in strain to cumulative anthracycline dose, age and clinical risk factors. Data were analyzed using SPSS version 15 (SPSS Inc, Chicago, Illinois), and considered significant if $p < 0.05$.



A)



B)

Figure 4.1 2D myocardial strain measurement, longitudinal (A) and radial (B) myocardial strain measurements. The colored lines represent measurements of regional myocardial deformation; the dotted line on the longitudinal strain image represents their 'global' average (all taken from the 2D image at the top left).

Table 4.1 Participant characteristics

Number of participants	52
Age (years)	49 ± 9
Height (centimetres)	161 ± 5
Weight (kilograms)	76 ± 23
Side of breast cancer	
Right	28
Left	24
Both	0
Anthracycline type	
Doxorubicin (Dox)	40 (77%)
Max. Dox dose (mg/m^2)	318
Mean Dox dose (mg/m^2)	236 ± 33
Epirubicin (Epi)	12 (23%)
Max. Epi dose (mg/m^2)	581
Mean Epi dose (mg/m^2)	408 ± 110
Risk factors	
IHD	3 (6%)
Hypercholesterolemia	11 (21%)
Smoking	13 (25%)
Hypertension	13 (25%)
Diabetes	2 (4%)

IHD (ischemic heart disease)

4.3 RESULTS

All 52 participants recruited into the study had a baseline echocardiogram before chemotherapy and a follow-up echocardiogram immediately after completing chemotherapy. Four (n = 34) or six cycles (n = 18) of anthracycline chemotherapy (doxorubicin or epirubicin) was administered, as determined by the oncology team. Patient demographics and clinical characteristics are presented in table 4.1.

Measurement of biplane LVEF was possible in 50 of the 52 participants (96%). LV myocardial systolic strain measurements were feasible in 47 of the 52 (90%) and before chemotherapy strain measurements were consistent with previously reported normal measurements (Marwick, 2009). Paired measurements of longitudinal, radial and circumferential strain were possible in 41 of these 47 (87%). Limited image quality before and/or after chemotherapy meant LVEF and strain measurements were not possible in all participants (image quality was limited most often by left sided mastectomy and/or breast implant).

No participant reported symptoms of cardiac failure during the time of follow-up. No significant difference in the LV dimensions before and after chemotherapy was observed. A statistically significant reduction in the average LVEF after chemotherapy was observed (table 4.2); however, no participant had a reduction in LVEF of ≥ 10% after treatment. In fourteen participants LVEF fell to below 55%. In one participant LVEF was < 50% after chemotherapy; in this case LVEF dropped from 56% before to 47% after chemotherapy.

Global longitudinal 2D strain after chemotherapy was significantly reduced; the average global longitudinal strain value dropped from -17.8% to -16.3% ($p < 0.01$ (see table 4.2)). In 21 of the 44 participants (48%), global longitudinal strain was reduced by > 10% from before chemotherapy, and reduced in 7 of the 44 participants (16 %) by > 20%. Longitudinal strain after chemotherapy was reduced by greater than the mean minus 1SD of the baseline strain measurement in 12 of 44 (27%) and by greater than the mean minus 2 SD in 6 out of 44 participants. In a subgroup, longitudinal strain was measured as a global composite from the apical 4, 2 and long axis views. A similar reduction of > 10% global longitudinal strain was observed in this measurement in 12 of 19 patients (table 4.3). Participants with an LVEF ≤55% after anthracycline chemotherapy (but not reduced by >10%), had significantly reduced global longitudinal strain compared to those with LVEF after chemotherapy of >55% ($p \leq 0.01$).

Table 4.2 LVEF and global strain measurements before and after chemotherapy

	Before	After	Percentage with 10 – 19 % reduction	Percentage with > 20 % reduction
LVEF (%)	58.6 ± 2.6	56.0 ± 2.8 †	0	0
Global longitudinal strain (%)	- 17.8 ± 2.1	- 16.3 ± 2.0 †	48 %	16 %
Global radial strain (%)	40.5 ± 11.4	34.5 ± 11.4 †	59 %	46 %
Global circumferential strain (%)	- 20.3 ± 2.6	- 20.3 ± 3.3	32 %	6 %

(Values expressed as mean ± SD, † p <0.01 vs. before value)

Global radial strain after chemotherapy was also significantly reduced; the average global radial strain dropped from 40.5% before to 34.3% ($p < 0.01$) after treatment (see table 4.2). In 24 of the 41 participants (59 %) global radial strain was reduced by > 10% after chemotherapy, with radial strain reduced being reduced in 19 of the 41 participants (46%) by > 20% (table 2). Similar to longitudinal strain, a reduction after chemotherapy of more than the mean minus 1SD of the before treatment measurement was observed in 16 of 41 participants (39%). In contrast, global circumferential LV strain after chemotherapy remained largely unchanged from beforehand (table 4.2), only three participants' (6%) global circumferential strain was reduced by ≥ 20% after chemotherapy.

Table 4.3 Subgroup (triplane) analysis of longitudinal strain before and after anthracycline chemotherapy

n =19	Before	After	Percentage with 10 – 19 % reduction	Percentage with > 20 % reduction
LVEF (%)	58.4 ± 2.4	56.0 ± 2.5 †	0	0
Global triplane longitudinal strain (%)	- 18.5 ± 1.6	- 16.6 ± 1.6 †	58 %	11 %

(Values expressed as mean ± SD, † p <0.001 vs. before value)

Regional analysis of longitudinal LV 2D strain revealed significant reductions in all but the apical lateral segment after chemotherapy (see table 4.4). Regional analysis of radial strain revealed significantly reduced measurements in 3 of the 6 regions (the anteroseptal, septal and inferior segments) after chemotherapy (see table 4.5). However, regional analysis of circumferential strain showed a significant reduction in the septal segment only (see table 4.6).

Table 4.4 Regional longitudinal strain values before and after chemotherapy

	Basal septum	Mid septum	Apical septum	Apical lateral	Mid lateral	Basal lateral
Before	-19.2 % ± 5.5	-21.0 % ± 4.5	-22.4 % ± 4.7	-14.7 % ± 6.4	-15.2 % ± 6.2	-18.5 % ± 5.9
After	-17.1 % ± 5.4 *	-19.3 % ± 5.2 †	-20.9 % ± 5.4 †	-13.6 % ± 6.9	-13.9 % ± 6.8 †	-16.6 % ± 6.1 †

** p ≤0.001 vs. before value, † p <0.05 vs. before value*

Table 4.5 Regional radial strain values before and after chemotherapy

	Anterior septum	Anterior	Lateral	Posterior	Inferior	Septal
Before	39.4 % ± 14.1	36.2 % ± 13.9	36.6 % ± 13.0	40.0 % ± 13.7	43.8 % ± 14.3	45.3 % ± 15.0
After	32.8 % ± 12.2 †	32.9 % ± 12.8	34.9 % ± 14.8	35.3 % ± 14.8	35.4 % ± 13.6 †	36.2 % ± 12.6 *

** p ≤0.01 vs. before value, † p <0.05 vs. before value*

Table 4.6 Regional circumferential strain values before and after chemotherapy

	Anterior septum	Anterior	Lateral	Posterior	Inferior	Septal
Before	-22.5 % ± 7.7	-19.4 % ± 7.5	-16.5 % ± 6.8	-17.2 % ± 6.9	-22.3 % ± 5.7	-26.5 % ± 5.7
After	-22.6 % ± 6.6	-19.8 % ± 6.5	-16.6 % ± 6.2	-18.0 % ± 6.3	-21.8 % ± 5.6	-24.0 % ± 6.3 *

* *p <0.05 vs. before value*

Variation in peak LV systolic strain between regions and also within regions was observed. This variation was greatest in the radial plane and least in the circumferential plane, as demonstrated by greater radial strain standard deviations. In addition to global and regional strain, peak systolic strain rate was measured, although no significant reduction was observed in systolic strain rate between paired measurements (table 4.7).

Table 4.7 Systolic strain rate analysis before and after anthracycline chemotherapy

	Before	After	*p* value
Longitudinal strain rate (s^{-1})	-0.87 ± 0.14	-0.84 ± 0.12	0.15
Radial strain rate (s^{-1})	2.20 ± 0.56	2.02 ± 0.52	0.13
Circumferential strain rate (s^{-1})	-1.72 ± 0.34	-1.73 ± 0.28	0.54

Values expressed as mean ± SD

The type of anthracycline and cumulative anthracycline dosage were recorded for all participants. The maximum doxorubicin dose was 318mg/m^2 and the maximum epirubicin dosage was 581mg/m^2. No significant correlation between anthracycline type or dosage and reductions in LVEF or systolic strain was found.

Chi square analysis was used to assess the impact of baseline clinical risk factors (hypertension, diabetes, cholesterol, smoking history, and history of ischemic heart disease) and reduced echocardiographic LV systolic strain measurements at follow up. No significant association between cardiac risk factors and the reduction in strain measurements was observed when tested individually or together; there was no significant difference in blood pressure measurement before and after chemotherapy. Univariate analysis between age, clinical risk and cumulative anthracycline dose to change in global strain before and after treatment showed no significant correlation between any of the parameters examined.

The interobserver and intraobserver differences in measurement of strain were similar (interobserver vs. intraobserver); for global longitudinal strain, the mean interobserver difference was -1.73 ± 1.0 and the mean intraobserver difference was -0.86 ± 0.59. For global radial strain, the mean interobserver difference was 5.0 ± 7.80 and the mean intraobserver difference was 3.40 ± 12.40. For global circumferential strain, the mean interobserver difference was 1.48 ± 1.24 and the mean intraobserver difference was 1.62 ± 1.10.

4.4 DISCUSSION

The principal findings of this study suggest that myocardial strain imaging may be more sensitive than LVEF in detecting changes in LV systolic function early after therapeutic doses of anthracycline chemotherapy. Greater than 10% reductions in peak longitudinal and peak radial LV systolic strain measurements were observed after chemotherapy in ~ 50% of participants. Twenty-seven percent of these had a reduction in longitudinal strain, and 39% had a reduction in radial strain more than the mean minus 1 SD from baseline. No corresponding reduction in LVEF greater than or equal to 10% was observed in any participant after chemotherapy. Furthermore, participants with an LVEF ≤55% after anthracycline chemotherapy had significantly reduced global longitudinal strain compared with those with an LVEF >55%. While these observations suggest that longitudinal and radial strain identify early impairment of myocardial systolic function, radial strain measurements had high variability; determining the significance of the observed reductions will require long term follow up before strain is used with established markers of cardiotoxicity in decisions related to changes in therapy.

LV muscular anatomy is complex; overall it has a helical configuration, with 3 'layers' within the ventricular wall. The muscle 'layers' do not comprise discrete muscle groups, but are classified according to the predominant orientation of myocytes (Anderson, Ho, Redmann, Sanchez-Quintana, & Lunkenheimer, 2005). LVEF is the current standard method for monitoring systolic function in chemotherapy patients; however LVEF relies upon geometric assumptions for its calculation (McGowan & Cleland, 2003). Moreover, LVEF is a measure of global function that is unable to determine which muscle layer is affected. In contrast, strain imaging can measure both regional and global function and does not rely on assumptions about cardiac geometry. The contemporary 2D speckle tracking strain imaging technique is distinct from the older 'tissue' Doppler-based technique, as it is semiautomated, utilizes low frame rates, and importantly is relatively angle independent (Argyle & Ray, 2009).

We observed significant reductions in global longitudinal and radial systolic strain measurements after chemotherapy in the absence of any reported symptoms (table 4.2). Reduced global longitudinal strain with preserved LVEF has been previously reported in other disease settings (Koyama, Davidoff, & Falk, 2004; Serri et al., 2006; Weidemann et al., 2003). Reduced longitudinal and radial strain using the older tissue Doppler-based strain technique has been previously reported in a small study of 16 patients over the age of 65 after pegylated anthracyclines (Jurcut et al., 2008). A reduction in radial 2D strain has been reported in an animal

model early after anthracycline therapy (Migrino, et al. 2008). Our observation of reduced global longitudinal and radial strain early after anthracycline chemotherapy was made in a larger group of 52 patients and with the semiautomated 2D strain imaging technique.

Sawaya et al recently reported that reduced longitudinal and radial strain after 3 months of cancer treatment could predict the later development of cardiotoxicity while reduced LVEF after 3 months could not (Sawaya et al., 2011). However, their study participants were a heterogeneous group that included participants who had received anthracyclines, adjuvant therapy with trastuzumab, and thoracic radiotherapy. Therefore, our observations differ significantly for 2 important reasons; we studied patients after anthracycline treatment only (with no radiotherapy or adjuvant trastuzumab), and observed reduced longitudinal and radial strain 1 week following anthracycline therapy. It must be noted however, that the variability of radial strain measurements using currently available techniques make it difficult to determine if the reductions observed in radial strain after chemotherapy represent a true reduction or can be accounted for by the variability of the measurement.

In contrast to longitudinal and radial strain, global circumferential systolic strain remained largely unchanged after chemotherapy (table 4.2). Reduced longitudinal strain, with preserved circumferential strain and preserved LVEF in long-term cancer survivors treated with anthracyclines and/or radiotherapy was reported recently by Tsai et al (Tsai et al., 2011). Reduced longitudinal strain in long-term survivors treated with anthracyclines and a subgroup treated with adjuvant trastuzumab was also reported recently by Ho et al (Ho et al., 2010). We additionally observed a reduction in radial strain parameters, suggesting a more generalized myocardial involvement consequent to oxidative stress mediated anthracycline chemotherapy.

Fallah-Rad et al recently reported significantly reduced longitudinal and radial strain after anthracyclines in participants who later developed trastuzumab-induced cardiomyopathy, but did not report any circumferential measurements (Fallah-Rad et al., 2011). In our study, global circumferential LV strain remained largely unchanged after chemotherapy. Paired analysis for within patient differences revealed global circumferential strain was reduced ≥ 20% in only 3 participants. Interestingly, the 1 participant with an important drop in LVEF to 47% after chemotherapy was 1 of these 3. While it is inappropriate to extrapolate an observation in a single patient, it suggests that changes in circumferential strain occur later than changes in the other 2 axes, and that larger reductions in LVEF may occur in association with significantly reduced circumferential strain.

Significant reductions in regional longitudinal and radial systolic strain after chemotherapy were observed (tables 4.4 and 4.5) in the LV lateral wall and most often in the septal regions, possibly indicating a regional heterogeneity in the development of systolic dysfunction. These novel findings suggest that identifying reduced strain in the LV lateral wall may be a way of monitoring myocardial function in this setting. Regional variation in peak LV systolic strain was observed, in keeping with observations from previous reports (Marwick, 2009; Serri et al., 2006) and is likely due to the variable LV fiber orientation shown to exist within and between subjects (Anderson et al., 2005). However, as 2D strain is dependent on image quality, some regional variation may be attributable to this as well.

Contrary to our observations related to strain, no significant reduction in global or regional systolic strain rate was observed after chemotherapy. This finding may indicate that the degree of systolic myocardial deformation is impaired prior to reduction in the rate at which deformation occurs. Alternatively, it may be that frame rates between 50 – 70 frames per second are not high enough to detect changes in strain rate.

In adults, heart failure induced by the anthracycline doxorubicin has been reported to occur in 4 - 5% at a cumulative dose of 500-550 mg/m^2, and up to 36% at a cumulative dose 600 mg/m^2 or more (Singal & Iliskovic, 1998; Swain, Whaley, & Ewer, 2003). Fortunately, dose-limiting strategies reduce cardiotoxicity; the incidence of heart failure is 1.6% with modern adjuvant therapy for breast cancer (doxorubicin between 240 and 360 mg/m^2) (Bird & Swain, 2008). However, microscopic analysis reveals that myocardial damage occurs with doses of doxorubicin as low as 180 mg/m^2 (although the precise mechanism responsible for this damage is unknown (Frei, 2008; Friedman, Bozdech, Billingham, & Rider, 1978; Gianni et al., 2008)) and support our observation of reduced LV systolic strain early after therapeutic doses of anthracycline chemotherapy

In keeping with previous reports, our results indicate that clinically significant reductions in LVEF (LVEF reductions >10% or absolute reductions in LVEF to < 50%) following low dose anthracyclines are rare. Our results also indicate that strain may detect early impairment of myocardial systolic function. The clinical relevance of these findings will require longer-term follow up for future cardiovascular events in this patient group.

Early initiation of heart failure treatment appears important for the recovery of LV function in patients who experience a significant reduction in LVEF due to anthracyclines (Cardinale et al.,

2010). Therefore, early identification of impaired systolic function is vital. Furthermore, given that many breast cancer patients first treated with anthracyclines will then receive trastuzumab, identifying patients at greater risk of developing cardiotoxicity is of considerable advantage. Early identification of those with significant reductions in strain (even in the absence of reduced LVEF) would enable targeted monitoring, together with the institution of supportive therapy with angiotensin-converting-enzyme inhibitors (ACEI's) or beta-blockers should further treatment with trastuzumab be required.

No significant association between cardiac risk factors (see methods) when tested individually or together and reduced global strain was found; no difference in blood pressure measurements before and after treatment was observed, however in order to confirm a lack of association between these cardiovascular risk factors and reduced strain measurements, larger studies of more participants with coexistent morbidities would be required. Only one participant in this study had a post chemotherapy LVEF < 50%. Larger scale studies over an extended period are required in order to determine the clinical relevance of the observed early reduction in strain measurements.

Our present study has some limitations. It was performed in a relatively small group of patients and does not provide longer-term follow up on the clinical implications of early anthracycline induced changes in myocardial strain. Our study does not include another imaging modality, such as magnetic resonance imaging, or the serial evaluation of cardiac biomarkers or ECG, which may provide additional information. Performance of biomarker testing was beyond the scope of the present study. Variability of strain measurements is an inherent limitation of the technique; however, our measurements are similar to other published data (Fallah-Rad et al, 2011; Ho et al, 2010; Marwick 2009). As the variability with radial strain is highest, despite the reduction in the mean value, it remains difficult to determine its real clinical value.

Our results indicate that 2D strain detects early reductions in global and regional systolic strain that may signify early impairment of myocardial systolic function immediately after anthracycline chemotherapy. Longer-term follow-up is needed to determine the clinical significance of the results of this study and whether early reduction in strain parameters result in the identification of future LV dysfunction in this clinical setting. Early identification of impaired systolic function will enable monitoring as well as targeted cardioprotective therapy in patients who require further (potentially life saving) treatment with trastuzumab.

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CHAPTER 5

ALTERED LEFT VENTRICULAR LONGITUDINAL DIASTOLIC FUNCTION CORRELATES WITH REDUCED SYSTOLIC FUNCTION IMMEDIATELY AFTER ANTHRACYCLINE CHEMOTHERAPY

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Preamble

During the analysis of LV systolic function made immediately after anthracycline treatment (presented in the previous chapter), it was evident that an analysis of LV diastolic function would be a logical extension of the research. Both phases of the cardiac cycle, systole and diastole, have complementary roles that ensure optimal cardiac performance: to date, monitoring cardiotoxicity has centered on measuring systolic function alone. The published findings from the analysis of diastolic function made at this initial time-point (immediately after anthracycline chemotherapy) are presented in the current chapter.

ABSTRACT

AIMS

The benefits from anthracycline chemotherapy are undermined by potentially life threatening cardiotoxicity. Transthoracic echocardiography is the most commonly used method for monitoring cardiotoxicity, and centers on the measurement of left ventricular systolic function. The aim of this study was to utilize two-dimensional speckle tracking echocardiography (2DSTE) at baseline and immediately after anthracycline chemotherapy to investigate whether patients with significant changes in systolic function after anthracycline therapy would also develop alterations in diastolic parameters.

METHODS AND RESULTS

Fifty-two women with histologically confirmed breast cancer were prospectively recruited. Echocardiograms were performed 1 week prior to and 1 week following chemotherapy (always before adjuvant trastuzumab or thoracic radiotherapy). Conventional Doppler, tissue Doppler imaging (TDI) and 2DSTE were used to measure diastolic function. 2DSTE measurements included longitudinal diastolic strain, early (E-Sr) and late (A-Sr) myocardial strain rate. 2DSTE and left ventricular ejection fraction (LVEF) were used to measure biplane longitudinal systolic function.

Altered LV diastolic function (including E-Sr) was observed in the entire cohort after chemotherapy, with a differential reduction in participants with a post therapy LVEF < 55%. Univariate predictors of E-Sr were LVEF post therapy ($p = 0.049$) and biplane systolic strain ($p = 0.01$). In a multivariate analysis, biplane systolic strain after chemotherapy was the strongest independent predictor ($p = 0.001$).

CONCLUSION

Altered LV diastolic function was observed immediately after administration of therapeutic doses of anthracycline chemotherapy. Furthermore, our analysis indicates that the changes in diastolic function are associated with reduced systolic function.

5.1 INTRODUCTION

Anthracycline agents are an essential part of breast cancer treatment due to their highly effective antineoplastic properties (Gianni et al., 2008). However, as anthracyclines are potentially cardiotoxic, close monitoring of cardiac function with their administration is mandatory (Bird & Swain, 2008; Singal & Iliskovic, 1998). Transthoracic echocardiography (TTE) is the most common method for monitoring cardiotoxicity (Jurcut et al., 2008a), and in this setting is centered on measuring left ventricular (LV) systolic function. Yet, both components of the cardiac cycle, systole and diastole are essentially linked and together must function normally for optimal cardiac performance (Lester et al., 2008).

Conventional Doppler measurements have demonstrated changes in diastolic function 6 years after childhood anthracycline chemotherapy (Dorup, Levitt, Sullivan, & Sorensen, 2004), and tissue Doppler imaging (TDI) measured 3 months and then 3 years after anthracyclines in a small adult population, revealed persistently reduced E' velocities (Tassan-Mangina et al., 2006). Strain imaging may enhance the evaluation of diastolic function (Nagueh et al., 2009; Zile & Brutsaert, 2002); however, there is a lack of published literature to support this. Previously, we reported reduced LV systolic strain immediately after anthracyclines (Stoodley et al., 2011), our aim in this study was to investigate LV diastolic function using conventional Doppler, TDI, strain and strain rate before and immediately after anthracyclines. We hypothesized that those with significant changes in systolic function immediately following therapy would also develop changes in diastolic parameters.

5.2 METHODS

The Sydney West Area Health Service and the University of Sydney Research Ethics Committees approved the study, and written informed consent was obtained from all participants. Fifty-two consecutive anthracycline naïve women with histologically confirmed breast cancer were prospectively recruited. All echocardiograms were performed at a single site, although chemotherapy was administered at one of four sites (depending on proximity to the patient's residence). The cumulative anthracycline dose was documented.

Clinical history, physical examination and echocardiographic characteristics were used to establish eligibility. Exclusion criteria included LV ejection fraction (LVEF) <50% prior to chemotherapy, rhythms other than sinus, more than mild valvular stenosis or regurgitation, prosthetic valve, previous ischemic heart disease or pacemaker. Height was measured at baseline, while weight and blood pressure were measured at the time of each echocardiogram. A detailed cardiac history, including clinical risk factors for heart disease (hypertension, diabetes, cholesterol, smoking and family history) was obtained at recruitment. Participants were also evaluated for any cardiac symptoms especially those of heart failure.

The initial echocardiogram was performed 1 week prior to the commencement of chemotherapy. The follow-up echocardiogram was performed 1 week following completion of chemotherapy (i.e. 12 or 18 weeks after the first study, depending on whether 4 or 6 cycles of chemotherapy were administered) and always performed before commencement of trastuzumab or thoracic radiotherapy. Echocardiograms were performed by two accredited sonographers using a Vivid 7 digital ultrasound system (GE medical systems, Norway) with a 2.5MHz variable frequency transducer optimized for image quality.

All TTE's were performed with patients in the left lateral position and images were obtained from the parasternal, apical and subcostal views. The transmitral early (E) and late (A) peak diastolic filling velocities, E/A ratio, E wave deceleration time and A wave duration were measured using conventional pulsed wave Doppler echocardiography with the sample volume placed at the mitral leaflet tips in the apical 4 chamber view. The velocity time integral of the A wave was measured and the atrial emptying fraction estimated as the A wave velocity time integral divided by the total mitral inflow velocity time integral. Maximum left atrial (LA) volume (just prior to mitral valve opening) and minimum LA volume were measured using the biplane

method of disks, according to the European Association of Echocardiography recommendations (Lang et al., 2006) and indexed to body surface area.

Peak velocity and the velocity time integral of the systolic, diastolic and atrial components of pulmonary vein flow were measured with pulsed wave Doppler by placing the sample volume in the proximal 1 cm of the right upper pulmonary vein. The systolic fraction was calculated as the systolic wave velocity time integral divided by the total forward pulmonary vein flow velocity time integral. Isovolumic relaxation time was measured from the continuous wave Doppler LV outflow tract signal. Pulsed wave tissue Doppler imaging (TDI) was used to measure the early (E′) and late (A′) peak velocities from the septal annulus.

Two-dimensional speckle tracking echocardiography (2DSTE) was used to measure biplane global longitudinal systolic and diastolic strain from the apical 4 and 2 chamber views, with observers blinded to LVEF results. The LV regions of interest for strain and strain rate analysis were manually selected by marking the endocardial border in the apical 4 and apical 2 chamber views. The tracking quality of individual LV segments was identified prior to analysis using the software scoring table on the off-line measuring package. Tracking quality was overridden in segments with no more than 2 rejected segments where the observer deemed tracking quality to be clearly acceptable. Images with persistently unacceptable tracking quality (more than 2 segments) were excluded from the final analysis. Measurements were made from 3 consecutive cardiac cycles and then averaged in order to obtain global strain and strain rate results.

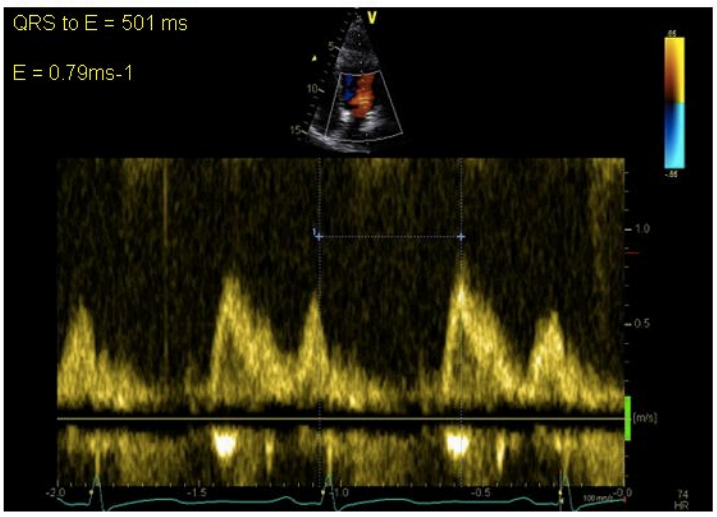


Figure 5.1 A)

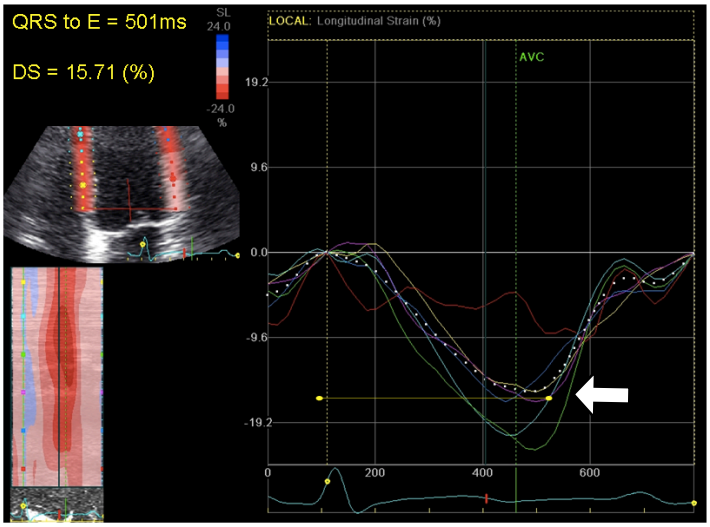


Figure 5.1 B)

Figure 5.1 Diastolic strain (Ds) measurement: calculation is made by measuring the time interval from the ECG R wave to the peak E velocity (figure A). This time interval is then applied to the 2DSTE trace to measure early global LV longitudinal diastolic strain (DS) from the dotted white line, as indicated by horizontal yellow line and the white arrow (figure B).

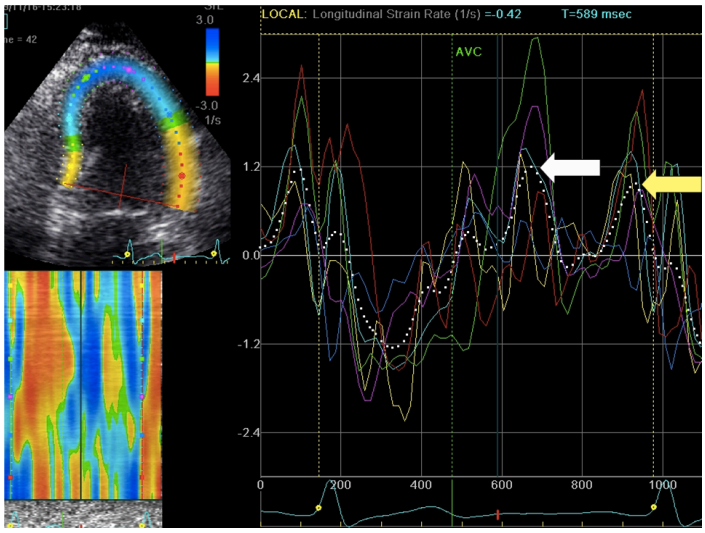


Figure 5.2 Global 2DSTE diastolic strain rate measurements: early peak diastolic strain rate (E-Sr) indicated by the white arrow and late peak diastolic strain rate (A-Sr) indicated by the yellow arrow. The coloured lines represent segmental measurements of strain rate and the dotted line the global average strain rate.

Systolic strain was measured as the peak negative strain during systole. Diastolic strain (DS) was estimated by measuring the time interval from the ECG R wave to peak E velocity with conventional transmitral Doppler echocardiography. The same time interval was applied to the 2DTSE trace to measure early global LV longitudinal DS as previously described (Dokainish, Sengupta, Pillai, Bobek, & Lakkis, 2008) (see figure 5.1). Heart rate was largely unchanged during both measurements. E/DS was calculated as a measure of elevated LV end diastolic pressure (Dokainish et al., 2008). 2DSTE was also used to measure biplane longitudinal diastolic strain rate. Global peak velocities in early (E-Sr) and late (A-Sr) diastole were measured with reference to the ECG (see figure 5.2).

Fifteen participants were randomly selected to determine interobserver and intraobserver variability in E-Sr and A-Sr, with the differences reported as mean difference ± standard deviation. One observer performed repeated measurements of E-Sr, A-Sr on a different occasion in order to measure intraobserver variability. Interobserver and intraobserver variability for systolic longitudinal strain has been previously reported (Stoodley et al., 2011).

All values were expressed as a mean ± SD unless otherwise stated. Paired t-tests were used to compare conventional Doppler, TDI and 2DSTE parameters before and after chemotherapy in the entire cohort (and within subgroups) to compare within patient changes. Linear regression and Chi square analysis were performed to determine univariate determinants of E-Sr as appropriate. Univariate variables with a significant correlation were entered into a stepwise multiple regression analysis to determine independent predictors of E-Sr. Data were analyzed using SPSS version 19 (SPSS Inc, Chicago, Illinois), and considered significant if $p < 0.05$.

5.3 RESULTS

All 52 participants had a baseline TTE before chemotherapy and a follow-up TTE 1 week following completion of chemotherapy. Limited image quality, most often due to left-sided mastectomy and/or breast implant, before and/or after chemotherapy meant that all measurements were not possible in all participants. Three participants (6%) had a history of ischemic heart disease, 11 (22%) had hypercholesterolaemia, 6 (12%) were smokers, 13 (26%) had hypertension and 2 (4%) were diabetic. Four (n = 34) or six (n = 18) cycles of anthracycline chemotherapy (doxorubicin or epirubicin) were administered, as determined by the oncology team. No participant reported symptoms or demonstrated signs of cardiac failure during follow-up. Maximum doxorubicin dose was 318mg/m^2 and maximum epirubicin dosage was 581mg/m^2 (see table 5.1).

Table 5.1

Participant demographics, cancer location and chemotherapy data

Number of participants	52
Age (years)	49 ± 9
Height (cm)	161 ± 5
Weight (kg)	76 ± 23
Side of breast cancer	
Right	28
Left	24
Anthracycline type	
Doxorubicin (Dox)	40 (77%)
Mean Dox dose (mg/m^2)	236 ± 33
Epirubicin (Epi)	12 (23%)
Mean Epi dose (mg/m^2)	408 ± 110

Paired measurements of conventional Doppler transmitral diastolic filling velocities were possible in 49 of the 52 participants (94%) and of pulmonary vein flow in 43 of the 52 participants (83%). Among the transmitral measurements, peak A ($p < 0.05$) and the atrial fraction ($p < 0.001$) were significantly increased, while the E/A ratio ($p < 0.05$) was significantly reduced after chemotherapy. Of the pulmonary vein flow measurements, systolic fraction was significantly increased ($p < 0.001$) and the diastolic velocity time integral (VTI) was significantly reduced ($p < 0.05$) after chemotherapy. LA maximum and minimum volumes and isovolumetric relaxation time did not change after chemotherapy (see table 5.2).

Paired measurements of TDI diastolic parameters measured at the septal annulus were possible in 46 of the 52 participants (88%). No significant change in E' velocity, the E/E' ratio or in A' velocity was observed after chemotherapy (see table 5.2). 2DSTE diastolic strain (DS) and early and late diastolic strain rate (E-Sr and A-Sr respectively) measurements were feasible in 45 of the 52 participants (87%). E-Sr was significantly reduced ($p < 0.01$) after chemotherapy, with an E-Sr reduction greater than the mean minus 1SD of the baseline measurement in 13 participants (25%). No significant change was observed in DS or E/DS (see table 5.2).

Previous analysis of LV systolic function in this cohort revealed significantly reduced global LV longitudinal systolic strain after chemotherapy (Stoodley et al., 2011). In order to investigate the relationship between reduced systolic function and diastolic function measurements after chemotherapy, participants were divided into two groups; group 1 ($n = 38$) comprised participants with an LVEF after chemotherapy > 55% and group 2 ($n = 14$) of those with an LVEF of < 55% after chemotherapy. Within group 1, only E-Sr was significantly reduced ($p= 0.03$) after chemotherapy. In contrast, within group 2, the A velocity and atrial fraction were significantly increased. The pulmonary vein atrial reversal duration minus the transmitral A duration was increased, almost reaching statistical significance ($p = 0.05$). The E/A ratio was significantly reduced. The diastolic VTI and atrial reversal duration decreased and systolic fraction increased significantly after chemotherapy in group 2. Moreover, E' and E-Sr were significantly reduced in group 2 (see table 5.3).

No significant association was present on Chi-square analysis for baseline clinical risk factors (hypertension, diabetes, cholesterol, smoking and family history) and reduced E-Sr post therapy. There was no significant difference in blood pressure measurements. As expected, linear regression analysis demonstrated a correlation with age and baseline LVEF and post therapy E-Sr ($r = -0.35$, $p = 0.04$ and $r = -0.54$, $p < 0.001$ respectively). Baseline systolic strain was also found to be

related to post chemotherapy E-Sr (r = -0.35, $p = 0.04$). No association was found between E-Sr post therapy and baseline LVEF, LV mass or cumulative anthracycline dose. Univariate predictors of post chemotherapy biplane E-Sr were LVEF ($p = 0.049$) and biplane systolic strain ($p = 0.01$); with age proving to be the strongest predictor (see figure 5.3 A and B). Multivariate predictors of biplane E-Sr post therapy were LVEF and biplane systolic strain, with post therapy biplane systolic strain being the strongest predictor (standardized coefficient β=-0.48, $p = 0.002$) (age was excluded from the multivariate analysis based on its established co-dependence with diastolic function).

Table 5.2 Conventional Doppler, tissue Doppler imaging and 2D speckle tracking echocardiography measurements (2DSTE) of diastolic function in the entire study cohort before and after anthracycline chemotherapy

	Before	After
Doppler measurements		
Peak E velocity ($m \cdot s^{-1}$)	0.72 ± 0.15	0.70 ± 0.13
Peak A velocity ($m \cdot s^{-1}$)	0.66 ± 0.13	0.69 ± 0.15 *
E / A ratio	1.13 ± 0.30	1.05 ± 0.28 *
Deceleration time ($m \cdot s^{-1}$)	220 ± 36	225 ± 38
Atrial fraction	36.0 ± 6.9	38.4 ± 7.5 †
PV systolic VTI ($cm\ s^{-1}$)	15.8 ± 3.5	16.5 ± 4.7
PV diastolic VTI ($cm\ s^{-1}$)	11.5 ± 3.5	10.5 ± 2.5 *
Systolic fraction	57.5 ± 9.3	62.3 ± 8.5 †
PV atrial reversal duration (ms)	119 ± 19	116 ± 15
Tissue velocity imaging		
E′ velocity ($cm\ s^{-1}$)	8.3 ± 2.0	8.2 ± 4.7
E/E′ ratio	8.8 ± 2.3	9.2 ± 2.6
A′ velocity ($cm\ s^{-1}$)	9.8 ± 4.2	9.0 ± 1.8
2DSTE measurements		
Diastolic strain (%)	10.9 ± 2.5	10.4 ± 2.0
Early strain rate (ESR) (s^{-1})	1.00 ± 0.24	0.90 ± 0.22*
Active strain rate (ASR) (s^{-1})	0.63 ± 0.16	0.63 ± 0.16

** p <0.05 vs. before chemotherapy value, † p <0.01 vs. before chemotherapy value*

(where PV = pulmonary vein, VTI = velocity time integral)

Table 5.3 Measurements of systolic and diastolic function with significant variation after chemotherapy in group 2 (n = 14)

	Before	After
Systolic function		
Bi-plane longitudinal systolic strain (%)	-18.8 ± 2.81	-15.6 ± 2.47†
Diastolic function		
Peak A velocity (ms^{-1})	0.64 ± 0.14	0.69 ± 0.13*
E / A ratio	1.17 ± 0.28	1.04 ± 0.26*
Atrial fraction	35.8 ± 5.72	39.2 ± 5.45*
Diastolic VTI	12.76 ± 2.89	11.07 ± 2.53††
Atrial reversal duration	125 ± 37.2	117 ± 19.3*
E' velocity ($cm\ s^{-1}$)	8.24 ± 1.57	7.48 ± 1.43*
Early strain rate (E-Sr) (s^{-1})	1.04 ± 0.19	0.80 ± 0.17††

*†† $p < 0.01$ vs. before chemotherapy, † $p = 0.01$ vs. before chemotherapy, * $p < 0.05$ vs. before chemotherapy value*

Interobserver and intraobserver differences in the measurement of diastolic strain rate were calculated. For E-Sr, the mean interobserver difference was 0.08 ± 0.12 s^{-1} and the mean intraobserver difference was 0.01 ± 0.05 s^{-1}. For A-Sr, the mean interobserver difference was 0.06 ± 0.12 s^{-1} and the mean intraobserver difference 0.01 ± 0.08 s^{-1} (see figures 5.4 and 5.5). For global longitudinal systolic strain, the mean interobserver difference was -1.73 ± 1.0 and the mean intraobserver difference was -0.86 ± 0.59 (as previously reported (Stoodley et al. 2011)).

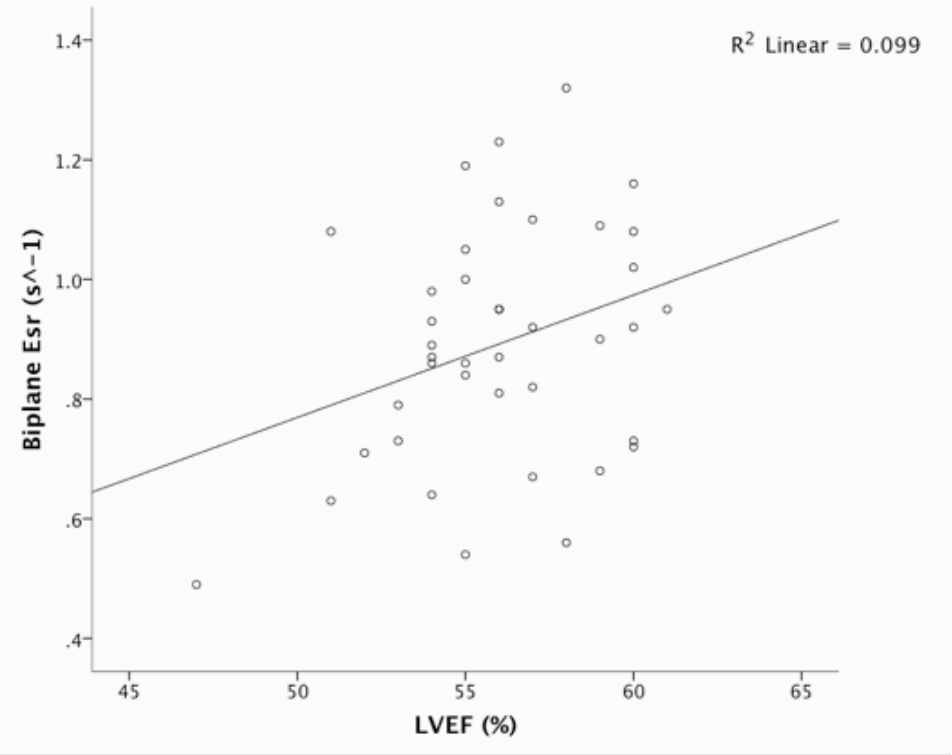


Figure 5.3 A) Pearson's linear regression analysis revealed a significant correlation between biplane ESR and LVEF post chemotherapy, $p = 0.049$.

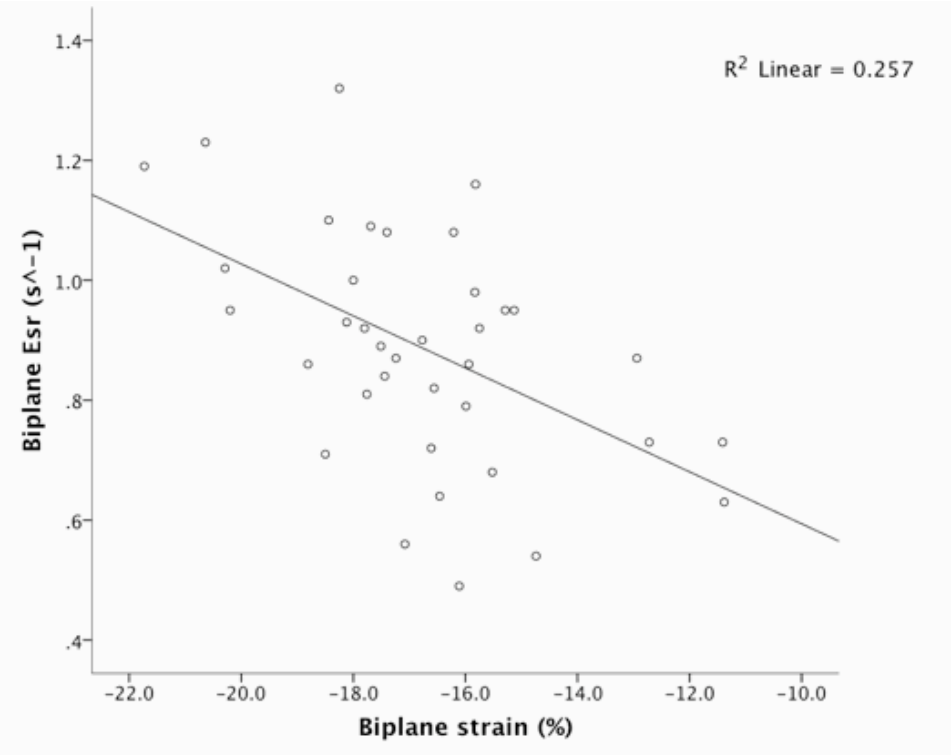


Figure 5.3 B) Pearson's linear regression analysis revealed a significant correlation between biplane ESR and peak longitudinal systolic strain post chemotherapy, $p = 0.01$.

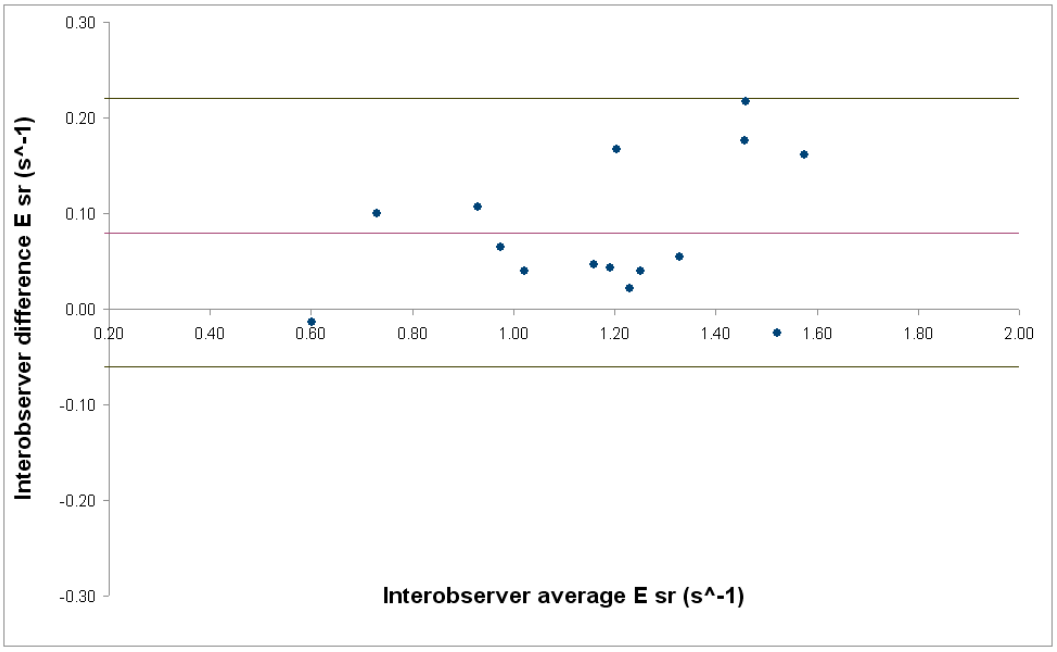


Figure 5.4 A) Bland Altman plot of interobserver differences in E-Sr.

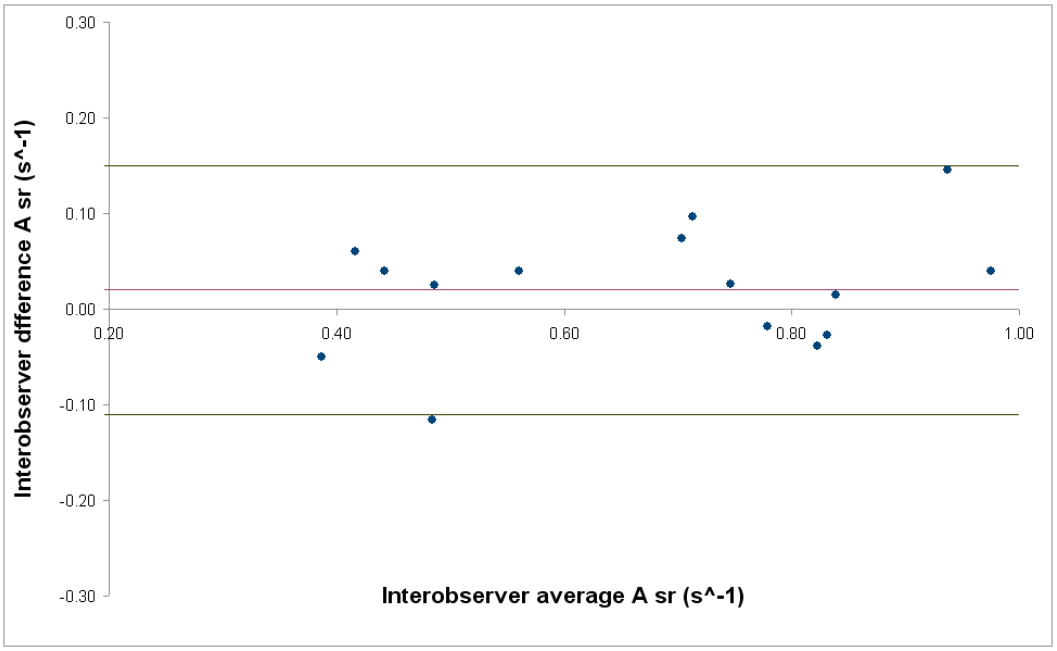


Figure 5.4 B) Bland Altman plot of interobserver differences in A-Sr. (**Note:** figures 5.4 A and B were published as supplementary material and available on-line only).

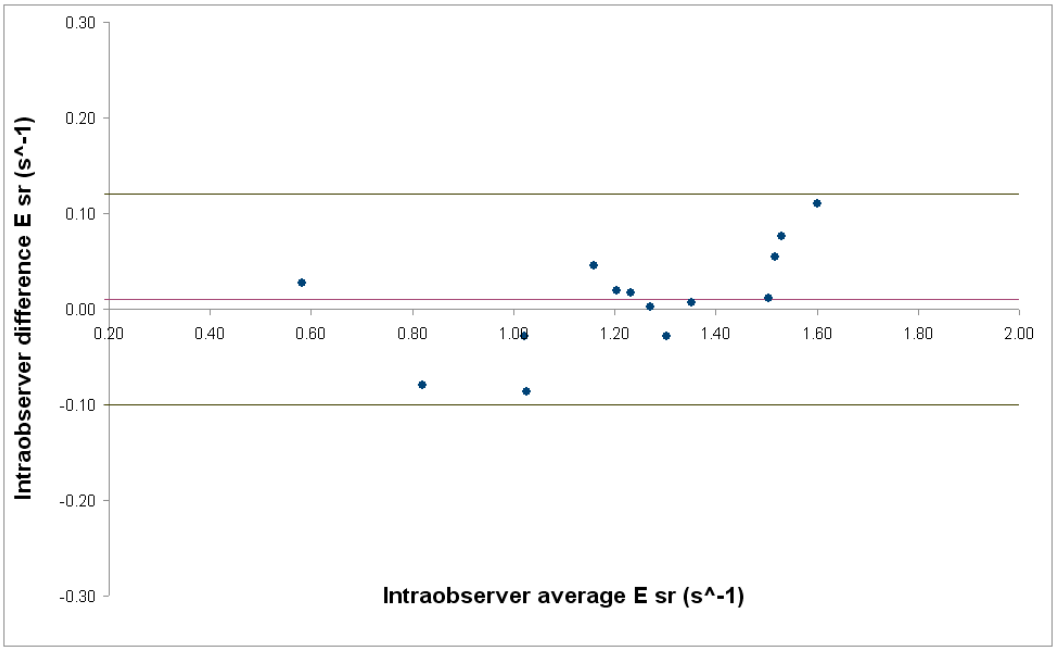
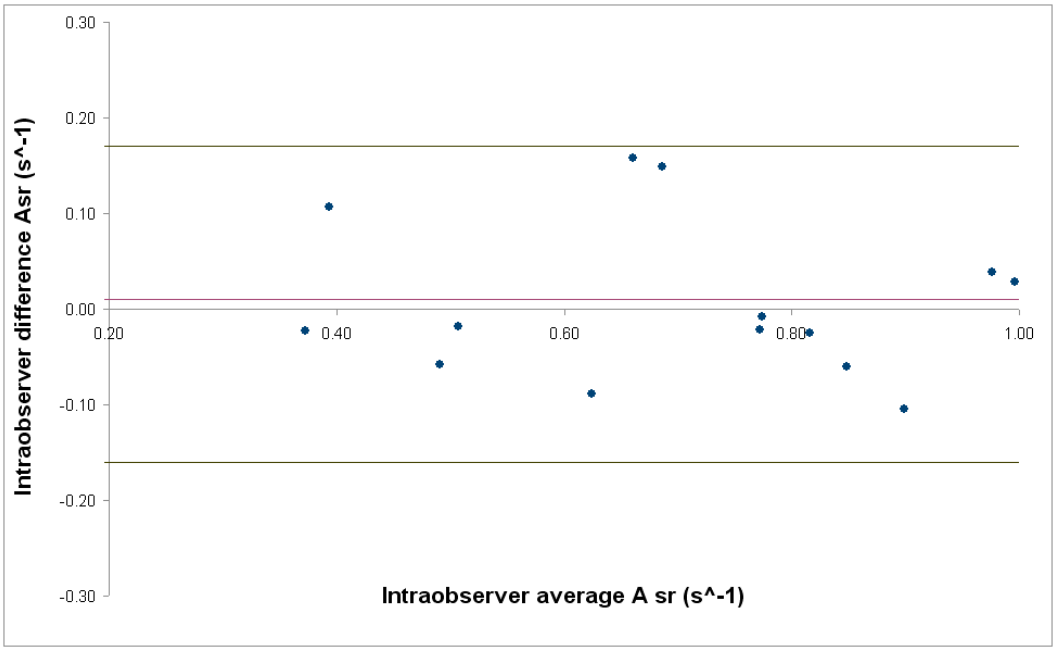


Figure 5.5 A) Bland Altman plot of intraobserver differences in E-Sr.



Figures 5.5 B) Bland Altman plot of intraobserver differences in A-Sr. (**Note:** images 5.5 A and B were also published as supplementary material and available on-line only).

5.4 DISCUSSION

Assessment of LV diastolic function is an essential part of a standard TTE examination. However, when monitoring cardiotoxicity, evaluation has typically focused on measuring LV systolic function by LV ejection fraction (LVEF). We previously reported reduced systolic function using strain imaging immediately after anthracycline chemotherapy (Stoodley et al., 2011); in the present study we observed alterations in diastolic function immediately after treatment. Reduced baseline systolic strain was found to be predictive of lower post chemotherapy E-Sr. Moreover, a differential reduction in diastolic function parameters in participants with LVEF < 55% after chemotherapy was observed.

Early identification of patients most likely to develop significant LV dysfunction following anthracyclines is of considerable clinical value, as it will enable targeted monitoring as well as cardioprotective therapy. Anthracycline-related diastolic dysfunction in paediatric (Dorup et al., 2004) and adult populations (Tassan-Mangina et al., 2006) in the intermediate and long term has been described. To our knowledge, our study is the first to report altered diastolic 2DSTE strain measurements and its association with systolic dysfunction immediately after anthracycline therapy: thus, our results provide an important insight to early myocardial changes following anthracyclines. Longer term follow up for the development of symptomatic heart failure is required to determine whether the early changes we have demonstrated will help identify patients at risk. For now, identifying altered diastolic function early may assist in the recognition and confirmation of anthracycline induced systolic dysfunction.

Using TDI to measure systolic and diastolic function is now routine in TTE examinations. Reduced TDI E' velocities 3 months after anthracycline chemotherapy that persisted at 3 years, with similar reductions in LVEF and S' velocity have been reported in a study comprising 20 patients (Tassan-Mangina et al., 2006). However, while the reduction in LVEF reached statistical significance in that study, it was not clinically significant (mean LVEF post therapy 56 ± 8%). In contrast, we demonstrate a differential reduction in E' and E-Sr measurements in patients with LVEF < 55% post chemotherapy (group 2). Ho et al recently reported reduced E velocity, E/A ratio and E' velocity in women treated with anthracyclines over a longer duration with subjects having been treated with anthracyclines up to 6 years earlier (Ho et al., 2010). They also reported reduced 2DSTE longitudinal and radial systolic strain, but failed to report a link between diastolic and systolic measurements.

The E/E' ratio is an accurate and widely used estimate of LVEDP, although it has been suggested that using 2DSTE to calculate E/DS may provide a more robust measure of LVEDP (Dokainish et al., 2008). In the present study, no significant change in E/E', DS, E/DS or A-Sr in the entire study population or in either of the subgroups was observed. The absence of change in E/E' or E/DS may reflect the fact that these parameters are altered with chronic disease rather than changes that occur acutely (as the follow up echocardiogram was performed within 1 week of completion of chemotherapy). It is unclear why changes in E-Sr precede changes in DS, but it is likely that the rate of myocardial deformation is altered prior to a reduction in the deformation per se. Isolated reduction in E-Sr with normal A-Sr levels has been reported in hypertension (Saghir, Areces, & Makan, 2007) and may represent early change in diastolic function.

In contrast to systole, diastole comprises two distinct phases. The first phase of diastole, active relaxation, occurs in a series of energy consuming steps that are dependent upon numerous cellular processes for normal function (Zile & Brutsaert, 2002). Alterations in myocardial collagen properties, abnormal calcium handling and an increase in ventricular fibrosis are known to result in reduced LV compliance and relaxation, and occur to a greater degree with advancing age (Gilbert & Glantz, 1989; Innelli, Sanchez, Marra, Esposito, & Galderisi, 2008; Martos et al., 2007; Tighe et al., 2003). The exact mechanism responsible for myocardial dysfunction as a result of anthracycline administration is unclear, however the myocardial damage is thought to be mediated by intracellular oxidative stress that results in mitochondrial dysfunction, apoptosis and myocyte necrosis (Zuppinger, Timolati, & Suter, 2007).

What is evident from this study is that a decline in early LV relaxation occurs as a result of anthracyclines. This decline can be measured one week after the completion of therapy, and results in a compensatory increase in the atrial contribution - similar to alterations in diastolic function that occurs with advancing age (Klein et al., 1994; Tighe et al., 2003). Reports that document an increased risk of morbidity and mortality in association with isolated diastolic dysfunction (Redfield et al., 2003) further validate the importance of our observation in a cohort of patients with a mean age of 52 years. Reduced baseline systolic strain was found to be predictive of lower post chemotherapy E-Sr. Moreover, measurements of early LV relaxation (E' and E-Sr) were differentially reduced in patients with LVEF < 55% post chemotherapy (group 2), and were strongly associated with reduced LV systolic strain.

Our study is limited by the relatively small number of participants, particularly for subgroup analysis, however within patient differences were still observed. The study reports

changes immediately after anthracycline chemotherapy and does not include longer-term follow-up on the clinical implications of early anthracycline induced changes in myocardial strain. Our study includes TDI E' measurements sampled only at the septal annulus; an average of septal and lateral E' velocity is lacking. The variability in E-Sr in this study although similar to previous reports, is an inherent limitation of strain measurements and therefore clinical decisions cannot be based on this parameter alone. Our study does not include another imaging modality or the serial evaluation of cardiac biomarkers, which may provide additional information, as performance of these additional tests was beyond the scope of the present study.

In the present study we observed changes in LV diastolic function using 2DSTE immediately after administration of therapeutic doses of anthracycline chemotherapy. Our analysis also indicates that the changes in diastolic function are related to reduced systolic function. Recognition of altered diastolic function could serve as an indicator of altered systolic function, thereby ensuring that patients who require closer monitoring when additional treatment with trastuzumab is required are identified. In the context of the future development of heart failure, our data support the need for longer-term follow-up with 2DSTE in an important clinical setting that requires close collaboration between oncology and cardiology.

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CHAPTER 6

LEFT VENTRICULAR SYSTOLIC FUNCTION IN HER2/NEU NEGATIVE BREAST CANCER PATIENTS TREATED WITH ANTHRACYCLINE CHEMOTHERAPY: A COMPARATIVE ANALYSIS OF LVEF AND MYOCARDIAL STRAIN IMAGING OVER 12 MONTHS

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Preamble

In the previous two chapters short-term findings were presented; that is, the findings from measurements made immediately before and immediately after anthracyclines. In this chapter, the published observations from participants followed for 12 months are presented. These results are the necessary extension of the earlier observations, and provide intermediate-term measurements of LV systolic function after anthracyclines.

In distinction to the previous two chapters, observations presented in this chapter were made in human epidermal growth factor receptor (HER2/neu) negative participants only. The majority of contemporary research utilizing strain imaging has focused on HER2/neu positive patients and the effects of anthracyclines plus trastuzumab. Therefore, in order to pursue novel research, LV systolic function in HER2/neu negative participants (treated with anthracyclines, but not trastuzumab) was undertaken in participants followed for 12 months.

ABSTRACT

AIM

Anthracycline agents are undermined by their cardiotoxicity. As life expectancy following treatment is greatly improved, techniques that ensure early detection and timely management of cardiotoxicity are essential. The aim of the present study was to evaluate left ventricular (LV) systolic function with LV ejection fraction (LVEF) and two-dimensional myocardial strain up to 12 months after anthracycline chemotherapy, specifically in HER2/neu negative breast cancer patients.

METHODS

Seventy-eight consecutive anthracycline naïve breast cancer patients were studied before and immediately after anthracycline chemotherapy. Fifty HER2/neu negative patients were studied over 12 months with serial echocardiograms at 4 time points. All patients were treated with standard regimens containing anthracyclines.

RESULTS

Global systolic strain was significantly reduced immediately after, and 6 months after anthracyclines (-19.0 ± 2.3% to -17.5 ± 2.3% ($p < 0.001$) and -18.2 ± 2.2% ($p = 0.01$) respectively). A non-uniform reduction in strain was observed each time with relative sparing of the LV apex. LVEF remained largely unchanged at both time points. Global strain normalised by 12 months in the majority of patients. Persistently reduced strain was observed in 16% ($n = 8$); these patients had a greater reduction in strain at 6 months (≤ -17.2%), and had received higher cumulative anthracycline doses.

CONCLUSION

Myocardial strain imaging is more sensitive than LVEF for the early detection and intermediate term monitoring of LV systolic function following anthracycline chemotherapy in HER2/neu negative breast cancer patients, and may aid in the development of improved monitoring protocols.

6.1 INTRODUCTION

A greater understanding of tumor biology has helped to improve breast cancer treatment; in fact with current treatment regimens, the prognosis for many breast cancer patients is very favorable (Jemal et al., 2011). Yet, four decades after their development, anthracycline chemotherapy remain the cornerstone of breast cancer treatment (Burstein et al., 2012; Gianni et al., 2008). Anthracyclines possess potent anti-tumor properties, and their benefits are confirmed by a significant body of evidence ((EBCTCG), 2005; Gianni et al., 2009). However, their efficacy is undermined by dose dependent cardiotoxicity (Singal & Iliskovic, 1998; Singal, Li, Kumar, Danelisen, & Iliskovic, 2000; Zuppinger, Timolati, & Suter, 2007).

Although dose-limiting strategies reduce cardiotoxicity, a small number of patients still develop cardiac dysfunction. Management of cardiotoxicity is further confounded by its temporal variability, as well as the need in some cases to supplement anthracycline chemotherapy with adjuvant radiation and trastuzumab therapy, which are also potentially cardiotoxic (Bird & Swain, 2008; Slamon et al., 2001). Moreover, clear guidelines for the detection and management of cardiac dysfunction after anthracyclines are lacking (Wells & Lenihan, 2010).

Previously, we reported reduced left ventricular (LV) systolic function using myocardial strain imaging, in the absence of clinically significant changes in LV ejection fraction (EF), within 7 days of completing anthracycline chemotherapy (Stoodley et al., 2011). The primary aim of the present study was to evaluate LV systolic function in HER2/neu negative breast cancer patients up to 12 months after anthracyclines: we hypothesized that strain imaging would reveal LV systolic dysfunction not discernible with LVEF, and provide a means for identifying patients at risk of developing overt changes in systolic function.

6.2 PATIENTS AND METHODS

Seventy-eight consecutive anthracycline naïve patients with histologically confirmed breast cancer were prospectively recruited over 2.5 years. Recruitment commenced in October 2008 and ended in March 2011. All participants were treated with standard regimens containing anthracyclines. Transthoracic echocardiograms and thoracic radiotherapy (RT) were performed at a single site, while chemotherapy was administered at one of four sites (depending on proximity to the patient's residence). The study was approved by the Sydney West Area Health Service and the University of Sydney Research Ethics Committees, and written informed consent was obtained from all participants.

Clinical history, physical examination and echocardiographic characteristics were used to establish eligibility (103 potential patients were referred during the recruitment period; 6 did not meet inclusion criteria, 9 elected not to participate and 11 were lost to follow-up). Exclusion criteria included previous anthracycline exposure, LVEF <50% prior to chemotherapy, rhythms other than sinus, more than mild valvular stenosis or regurgitation, prosthetic valve, ischemic heart disease or pacemaker. Height was measured at recruitment; weight and blood pressure were measured at the time of each echocardiogram. Cardiac history was obtained at recruitment, including clinical risk factors for heart disease (hypertension, diabetes and smoking history) and participants were evaluated throughout the study for any cardiac symptoms especially those of heart failure.

Echocardiograms were performed at 4 time points. A baseline exam was performed prior to the commencement of anthracycline chemotherapy (T1). The second exam was performed within 7 days of completing anthracycline therapy (T2), and always before the commencement of RT. Participants received four or six cycles of anthracycline chemotherapy (as determined by the oncology team), therefore the second exam occurred either 12 or 18 weeks after the first. The 3rd and 4th echocardiograms (T3 and T4) were performed at 6 and 12 months after the initial exam in HER2/neu negative participants only. All echocardiograms were performed by either of two experienced research sonographers using a Vivid 7 digital ultrasound system (GE medical systems, Norway) with a phased-array transducer.

Echocardiograms were performed with patients in the left lateral position and images were obtained from the standard parasternal, apical and subcostal views. Images from the parasternal short axis and apical 4 chamber views were used to measure LV dimensions and tissue Doppler S' velocity. LVEF was measured by Simpson's method according to the recommendations

of the European Association of Echocardiography (Lang et al., 2006), and measured at the time of acquisition. Depth adjusted two-dimensional (2D) images of the left ventricle from the apical 4 and 2 chamber views were acquired and stored for off-line measurement of biplane LV longitudinal peak systolic strain (LPSS) using 2D speckle tracking echocardiography (with EchoPac PC version 6.0, GE Healthcare, UK). Frame rates were optimized at the time of acquisition to ≥ 45 frames/ second. To ensure reproducibility and accuracy, systole was defined as the interval from aortic valve opening to closure, measured by pulsed wave Doppler sampling of blood flow in the LV outflow tract.

The LV regions of interest for longitudinal strain analysis were manually selected by marking the endocardial border at end systole in the appropriate imaging plane. Strain traces from each segment were inspected to confirm that peak systolic strain was measured prior to aortic valve closure and the tracking quality of all images was identified prior to analysis using the software scoring table on the off-line measuring package. Tracking quality was overridden in segments with no more than 2 rejected regions where the observer deemed tracking quality to be clearly acceptable. Images with persistently unacceptable tracking quality (more than 2 segments) were excluded from the final analysis. Strain measurements were made from 3 consecutive cardiac cycles and then averaged to obtain segmental LPSS. Global LPSS was calculated by averaging the 12 segmental values in the apical 4 and 2 chamber views (see figure 6.1).

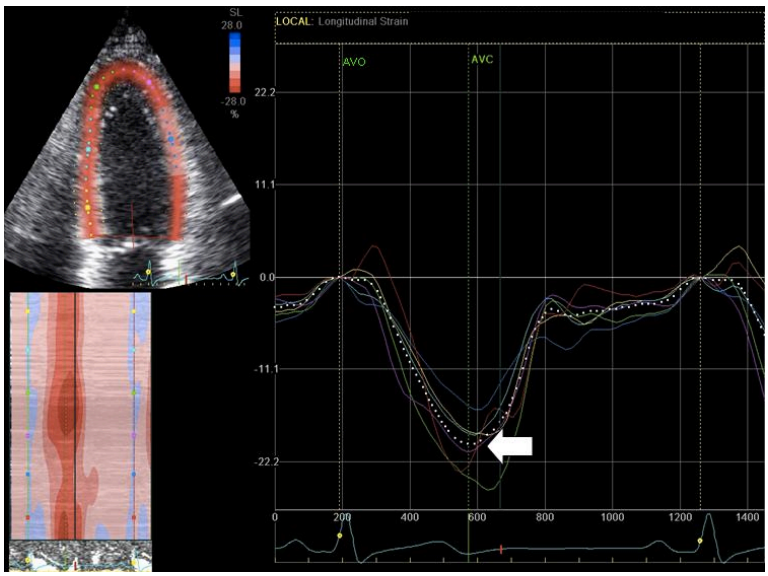

Figure 6.1 2D longitudinal myocardial strain measurement (%) from the apical 4 chamber view. The colored lines on the graph represent segmental deformation, the dotted line represents their 'global' average (global LPSS is indicated by the white arrow). Strain measurements made in the longitudinal plane are negative as myofibers shorten in the longitudinal plane.

Fifteen participants were randomly selected in order to calculate interobserver and intraobserver variability. LVEF and global LV LPSS measurements were repeated by a different observer (blinded to previous results) in order to measure interobserver variability. Intraobserver variability for LVEF and global LV LPSS were assessed by the same observer on a different occasion from the same digital data, using an offline system.

The mean and standard deviation (SD) was used to summarize each continuous variable. Chi squared tests were used to test for association between categorical variables. Mann-Whitney tests were used to test for differences in the distribution of continuous variables between two groups of patients. Linear mixed effects models were used to investigate within patient changes in LPSS and LVEF over four time points (in these models, time was considered as a factor with four levels corresponding to the four time points). Repeated measures ANOVA (with Bonferroni correction) was used to measure within patient changes in segmental LPSS, LV dimensions, tissue Doppler S' and heart rate. Data were analyzed using SPSS version 20 (SPSS Inc, Chicago, Illinois) and SPLUS version 8 (TIBCO Software, California) and considered significant if $p < 0.05$.

6.3 RESULTS

Seventy-eight participants (77 female and 1 male) with a mean age at recruitment of 52 ± 10 years were studied. Participants were treated with one of two regimens: Doxorubicin was used to treat 63 (81%) participants; the mean dose was 238mg/m^2 (range 140 - 340mg/m^2). Epirubicin was used to treat 15 (19%) participants; the mean dose was 392 mg/m^2 (range 255 - 572mg/m^2). All 78 participants had an echocardiogram at the first and second time points.

Measurement of biplane LVEF at T1 and T2 was possible in 76 of the 78 participants (97%). Mean LVEF at baseline (T1) was 58 ± 3% and within 7 days of completing anthracycline chemotherapy (T2) was 57 ± 3% ($p < 0.001$), a 2% relative reduction (with mean LVEF remaining within a clinically normal range). LV LPSS from the apical 4 chamber view was not possible in 9 of 78 (12%): left sided mastectomy was a limiting factor in 7 of the 9. Global biplane LV LPSS (from the apical 4 and 2 chamber views) at baseline was -18.6 ± 2.4% and within 7 days of completing anthracyclines was -17.0 ± 2.2% ($p < 0.001$), a 9% relative reduction.

Twenty-eight of the 78 participants (36%) were found to be HER2/neu positive by in situ hybridization, and therefore proceeded to treatment with trastuzumab following anthracycline therapy. These 28 participants were excluded from the analysis at T3 and T4. Fifty HER2/neu negative participants were studied at 4 time points over 12 months, all 50 (100%) had an echocardiogram at a minimum of 3 time points. Measurement of biplane LVEF was possible in 44 of the 45 (98%) who were followed for 12 months. The mean LVEF at each of the 4 time points is shown in table 6.1. A 2% relative reduction in LVEF from baseline was observed at T2 and T3. No significant changes in LV dimensions or systolic blood pressure were observed over the 12 month period (BSA was modestly reduced). A significant increase in heart rate ($p = 0.03$) was observed at T2; heart rate returned to its baseline value at 12 months. A significant reduction in tissue Doppler septal S′ velocity ($p = 0.01$) was seen after anthracycline therapy at T3 (see table 6.2 for demographic and standard echocardiographic measurements).

Table 6.1 LVEF and biplane global LV LPSS in HER2/neu negative patients before (T1), 7 days after (T2), 6 months (T3) and 12 months (T4) after anthracycline chemotherapy

(n = 50)	T1	T2	T3	T4
LVEF	58 ± 3%	57 ± 3%	57 ± 3% #	58 ± 3%
Global LV LPSS	- 19.0 ± 2.3%	- 17.5 ± 2.3% *	- 18.2 ± 2.2% #	- 19.1 ± 1.9%

*# p = 0.01, * p < 0.001*

Table 6.2 Demographic and standard echocardiographic measurements at 4 time points over 12 months

(n = 50)	T1	T2	T3	T4
BSA (m^2)	1.78 ± 0.22	1.77 ± 0.21	1.77 ± 0.21	1.75 ± 0.19 #
Heart rate (bpm)	73 ± 11	78 ± 10 †	75 ± 8	73 ± 9
Systolic BP (mmHg)	119 ± 12	117 ± 12	118 ± 11	120 ± 18
LVEDD (mm)	44 ± 4	44 ± 4	45 ± 4	44 ± 4
LVESD (mm)	31 ± 4	32 ± 2	32 ± 4	30 ± 3
Septal S′ (cm s^{-1})	8.2 ± 1.6	7.6 ± 1.0	7.3 ± 1.1 #	7.4 ± 1.2

† p < 0.05, # p = 0.01: T1 = 7 days before anthracyclines, T2 = 7 days after anthracyclines, T3 = 6 months after anthracyclines and T4 = 12 months after anthracycline chemotherapy, BP = Blood Pressure, bpm = beats per minute, LVEDD = left ventricular end diastolic dimension, LVESD = left ventricular end systolic dimension

Of the participants who were followed for 12 months, global biplane LV LPSS was possible on at least 3 of the time points in 42 of 45 (93%). LPSS could not be measured in 3 participants: left sided mastectomy ($n = 1$) and left sided lumpectomy ($n = 2$) were limiting factors. The global biplane LV LPSS at each of the 4 time points is shown in table 1. Significant reductions in global LV LPSS from baseline were observed at T2 ($p < 0.001$) and T3 ($p = 0.01$) in this extended follow-up group of HER2/neu negative participants (see Table 1).

Segmental strain analysis showed regional differences in the left ventricle following anthracycline chemotherapy: the basal and mid left ventricular segments were significantly reduced, whereas the LV apex was largely unchanged. A base to apex gradient was observed in LV

LPSS, with maximal strain values at the LV apex: a modest increase in this gradient was observed at all 3 time points (T2, T3, and T4) following anthracyclines (see table 6.3). Twelve months after anthracycline therapy, basal and mid segmental LV LPSS had normalized (see table 3 and figure 6.2).

Table 6.3 Longitudinal peak systolic strain (LPSS) values in the basal, mid and apical left ventricle at 4 time-points over 12 months

(n = 50)	T1	T2	T3	T4
Basal LV	-18.5 ± 2.4% [32%]	-16.6 ± 2.7% * [31%]	-16.9 ± 2.6% # [31%]	-17.9 ± 2.6% [31%]
Mid LV	-19.0 ± 2.7% [33%]	-17.2 ± 2.5% * [33%]	-17.4 ± 2.4% # [32%]	-18.2 ± 2.2% [32%]
Apical LV	-19.9 ± 4.1% [35%]	-18.9 ± 3.7% [36%]	-20.2 ± 3.5% [37%]	-21.2 ± 3.5% [37%]

*# p < 0.01, * p < 0.001 : T1 = 7 days before anthracyclines, T2 = 7 days after anthracyclines, T3 = 6 months after anthracyclines and T4 = 12 months after anthracycline chemotherapy; the values in parentheses represent the relative contribution of the basal, mid and apical segments to the overall LPSS at each time point.*

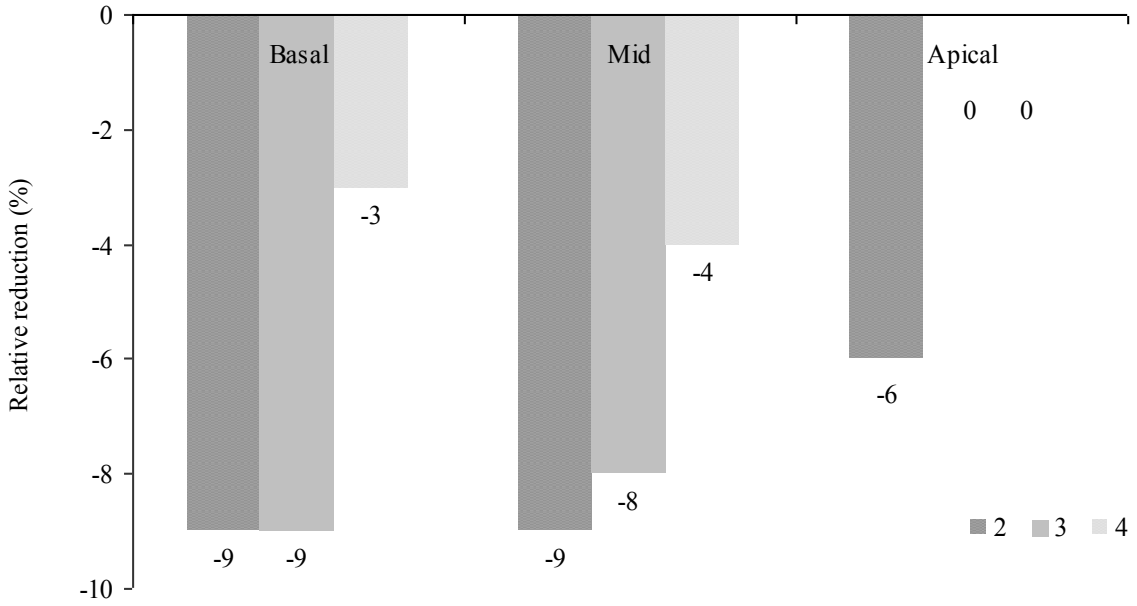

Figure 6.2 Percent relative reduction in global LV LPSS compared to baseline in the basal, mid and apical left ventricle; 2 = 7 days after anthracyclines, 3 = 6 months after anthracyclines, 4 = 12 months after anthracyclines.

Thirty-four of the 50 participants who were studied over 12 months received breast or chest wall radiotherapy (RT), with nodal irradiation as indicated. The standard radiation dose was 50 Gray (Gy) in 25 fractions. Twenty breast conservation participants received an additional dose to the tumor bed (10 Gy in 5 fractions). All RT was given after the completion of anthracycline chemotherapy. No significant reduction in LVEF or LV LPSS measurements was observed in participants who received RT compared to anthracyclines alone; no significant reduction was observed in the 15 participants who received left sided RT.

At twelve months, eight of the 50 HER2/neu negative group (16%) were found to have an LPSS ≥1 SD below the mean LPSS value (i.e ≤ 17.2%). Their mean LVEF showed no clinically significant change at the 4 time points (measuring 58 ± 2%, 56 ± 3%, 55 ± 2% and 57 ± 2% respectively). The mean global LV biplane LPSS at the 4 time points for these eight participants were -19.0 ± 1.8%, -16.8 ± 1.1%, -15.3 ± 1.0% and -16.6 ± 0.7% respectively, with significant reductions in LPSS compared to baseline noted at all 3 follow-up time-points ($p < 0.01$, $p < 0.001$ and $p < 0.01$). Furthermore, a low LV LPSS value at 12 months could be predicted by an LV LPSS value < -17.2% at 6 months with 100% sensitivity and 80% specificity (see figure 6.3). Only one participant had an LVEF measurement <50%, which was recorded at T3: LVEF measured 46% with a corresponding global LV LPSS measurement of -14.8%. An additional echocardiogram was performed at 9 months; LVEF and global LV LPSS at 9 and at 12 months measured 52% and -17.4% and 53% and -17.2% respectively.

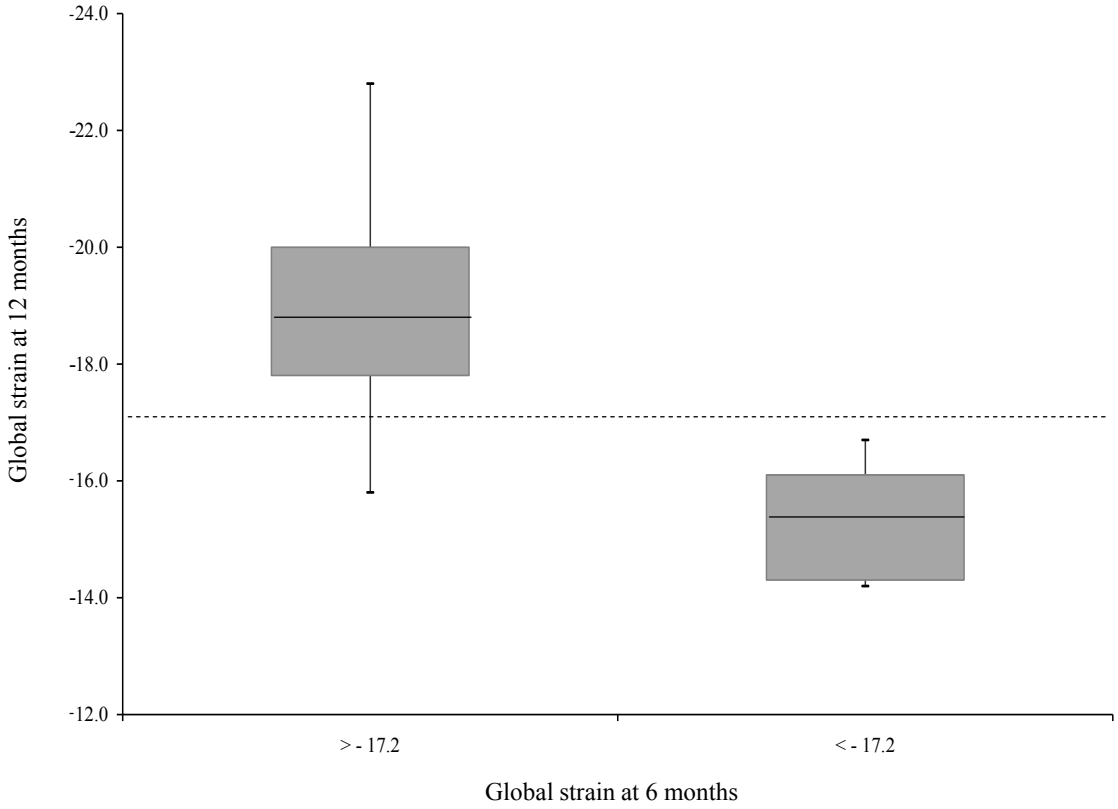

Figure 6.3 Box plot demonstrating the significant difference in global strain at 6 months in participants with a 12 month global strain value > -17.2% (n = 42) or ≤ -17.2% (n = 8).

Patients were divided into 2 groups based on whether their LV LPSS was > or ≤ -17.2% at 12 months (that is, -17.2% being 1 SD below the mean LV LPSS T4 value). The cumulative anthracycline dose for participants with a biplane global LV LPSS ≤ -17.2% at T4 was 318 ± 115mg/m^2, which although within therapeutic range, was significantly greater (p = 0.03) than the cumulative dose for those with an LPSS > -17.2 % (258 ± 75mg/m^2). The anthracycline agent (doxorubicin (75%) versus epirubicin (25%)) used was not significantly different between the two groups. There were no differences in age, BSA or smoking history between those with LV LPSS > or ≤ -17.2% at 12 months.

Nine HER2/neu negative participants were on treatment for hypertension (HT); three of the 9 had a biplane global LV LPSS ≤ -17.2% at 12 months and the presence of HT did not result in a persistently low LV LPSS of ≤ 17.2% at 12 months. Three of the 50 participants had diabetes (DM): 2 DM participants had a biplane global LV LPSS ≤ -17.2% at 12 months.

The interobserver and intraobserver measurements of LVEF and global LV LPSS revealed acceptable agreement. The coefficients of variation values were < 10%, which represents a range of variation similar to the range previously reported in the literature (see figure 6.4).

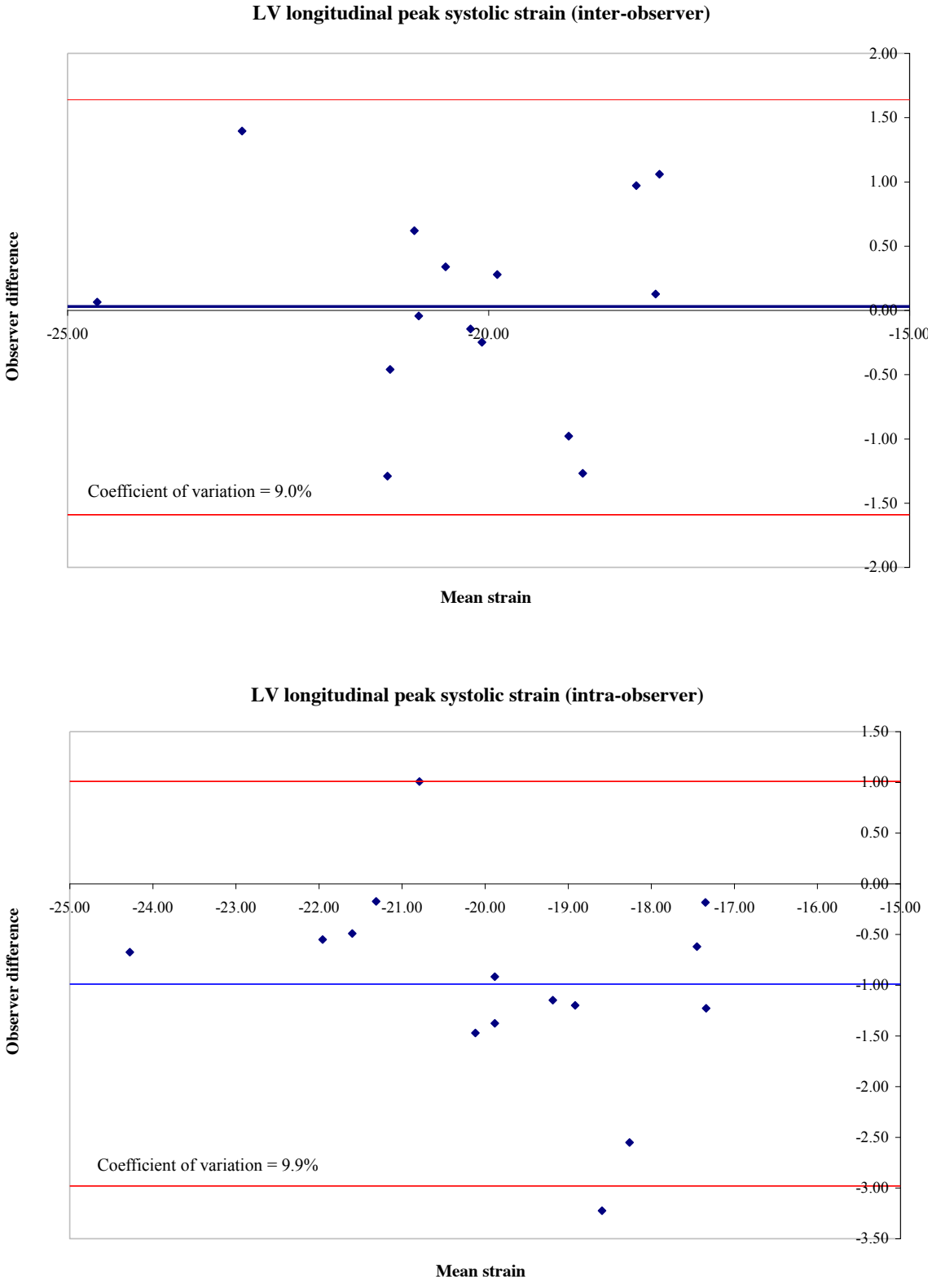

Figure 6.4 Bland-Altman figures of A) inter and B) intra-observer variation for global LV LPSS. (**Note:** these figures are published as appendix items, and available on-line only).

6.4 DISCUSSION

In the present study, we used LVEF and myocardial strain imaging, measured at 4 time points over twelve months, to evaluate LV systolic function in 50 consecutive HER2/neu negative breast cancer patients treated with anthracycline chemotherapy. The principal findings from this study indicate that: 1) myocardial systolic dysfunction can be detected with strain imaging within 7 days of completing anthracyclines without a similar discernible change in LVEF, 2) myocardial injury occurs non-uniformly within the LV with relative sparing of the apical segments, 3) in the majority of patients, low dose anthracycline induced myocardial injury is transient, without permanent dysfunction, and 4) persistently reduced global strain occurs in ~ 16% of HER2/neu negative patients when systolic strain is reduced ≤ -17.2% at 6 months following anthracycline therapy; this persistent reduction is associated with higher cumulative anthracycline doses. While there are several reports in the literature of chemotherapy related cardiotoxicity, ours is the first to focus on the effect of anthracyclines in HER2/neu negative patients over 12 months.

The observed reduction in LV LPSS within 7 days of completing anthracycline chemotherapy provides confirmation of observations we have reported previously (Stoodley et al., 2011): the current study involved a larger cohort. Approximately 30% of the cohort were HER2/neu positive, and reduced LV strain immediately after anthracyclines is particularly important for these patients, as they will require additional potentially cardiotoxic treatment with trastuzumab. A previous report demonstrated that longitudinal systolic strain is able to detect pre-clinical changes in LV systolic function in patients receiving trastuzumab in the adjuvant setting, whereas LVEF cannot (Fallah-Rad et al., 2011). Our results, which are consistent with these findings, highlight the sensitivity of strain to early changes in LV systolic function: a 9% relative reduction in global LPSS was observed immediately after chemotherapy, when the relative reduction in LVEF was only 2%.

Normally, regional LV myocardial systolic strain values increase modestly along the longitudinal axis from base to apex (Sengupta et al., 2007): we observed this characteristic pattern at baseline and after the administration of anthracycline chemotherapy. However, strain was not significantly reduced in the apical segments after anthracycline therapy. In fact, a greater difference between basal and apical strain values was evident after anthracyclines, indicating a non-uniform disruption of LV myocardial systolic function (table 6.3). A similar selective segmental disruption of systolic function has been reported in adolescent patients treated with anthracyclines (Poterucha, Kutty, Lindquist, Li, & Eidem, 2012), in HER2/neu positive patients

treated with trastuzumab with a variable history of anthracycline exposure (Sawaya et al., 2011), as well as in hypertrophic cardiomyopathy (Afonso et al., 2012).

Global LV LPSS normalized in the majority of participants at 12 months; however 16% (*n* = 8) were noted to have persistently reduced myocardial strain. In these participants, global LV LPSS was reduced by at least one standard deviation below the mean 12 month global strain value. Global strain in these participants prior to anthracycline chemotherapy was no different to the rest of the cohort; within 7 days of completing anthracyclines (T2), and at 6 months after anthracyclines (T3) their global strain was significantly reduced. Thus, global LV LPSS ≤ -17.2% at T3 was predictive (with 100% sensitivity and 80% specificity) of a 12 month (T4) strain value one standard deviation below the mean. Furthermore, analysis also showed that the cumulative anthracycline dose, although within the therapeutic range, was significantly higher in these participants.

Risk stratification protocols for the early detection of cardiotoxicity are important given increased life expectancy following breast cancer treatment. While HER2/neu positive patients treated with adjuvant trastuzumab are monitored closely, we have demonstrated subclinical LV dysfunction by strain analysis in HER2/neu negative patients, who comprise ~ 75% of all breast cancer patients. Thus, monitoring HER2/neu negative patients who receive anthracycline chemotherapy with echocardiograms that utilize strain imaging both at baseline and at 6 months following commencement of their treatment may help treating physicians identify patients with subclinical cardiac dysfunction who would benefit from additional cardiac monitoring and treatment. Furthermore, ensuring participants treated with higher cumulative anthracycline doses receive longer-term monitoring seems necessary. These are preliminary observations, which require longer-term follow-up, and may help in the development of risk stratification protocols for the early detection of cardiotoxicity, thereby improving management and outcomes in this patient population.

Recognizing the possible transient nature of LV systolic dysfunction after low dose anthracycline chemotherapy in HER2/neu negative patients is important. When treating patients with anthracyclines, oncologists aim to achieve an ideal anthracycline dosage; that is, a dosage that is therapeutic without being cardiotoxic (Eidem, 2008). While our study demonstrates a persistent reduction in LV strain in 16% of patients at 12 months, longer-term data are needed to provide confirmation of these observations in the longer term as well as their clinical relevance.

The present study is limited by its relatively small sample size. However, 16% of HER2/neu negative participants had reduced strain at 12 months. An additional imaging modality such as CMRI, which may have provided further information regarding LV systolic function was not included, nor were cardiac biomarkers. Left sided mastectomy or lumpectomy limit the biplane measurement of strain more than LVEF (which has been reported previously (Sawaya et al., 2011)). An examination of the efficacy of cardio-protective therapies (such as ACE inhibitors or beta blockers) used to treat LV dysfunction in this setting was beyond the scope of the current study, although future multi-centre studies have been planned. Furthermore, the development of an international registry, used to collect similar datasets, may be a way to advance research in this setting. Radiation therapy has been reported to augment the cardiotoxic effect of anthracycline chemotherapy (Tsai et al., 2011); we did not observe such an effect, which may be due to the relatively small cohort with a short duration of follow-up.

As is well known, anthracycline-induced cardiotoxicity results in changes to the structure and function of cardiac myocytes (Chen, Peng, Pentassuglia, Lim, & Sawyer, 2007; Sawyer, Peng, Chen, Pentassuglia, & Lim, 2010). Our findings in HER2/neu negative breast cancer patients indicate that myocardial strain imaging is a more sensitive method than the currently accepted measure of LV systolic function (LVEF) for the early detection and intermediate term monitoring of LV systolic function following anthracycline chemotherapy. The utilization of myocardial strain imaging may aid in the identification of those with preclinical LV systolic dysfunction: our data support the need for longer-term follow-up, where improved monitoring protocols and close collaboration between oncology and cardiology is essential.

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CHAPTER 7

TRASTUZUMAB INDUCED CARDIOTOXICITY: THE ROLE OF 2D MYOCARDIAL STRAIN IMAGING IN DIAGNOSIS AND MANAGEMENT

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Preamble

Modern treatment for breast cancer often involves a combination of anti-neoplastic agents. As such, anthracyclines are one of several agents used in the treatment of breast cancer. Trastuzumab is a contemporary biological agent that was introduced in chapter 3 part A of the thesis. As described therein, its introduction has led to an important advance in breast cancer treatment, however its use is also undermined by potential cardiotoxicity. The following published work details the findings of serial LVEF and strain measurements made over the course of approximately 18 months in a patient who developed heart failure during treatment with trastuzumab, which had been administered following completion of anthracyclines.

ABSTRACT

A 45 year-old female breast cancer patient developed heart failure during adjuvant trastuzumab therapy. Her initial left ventricular ejection fraction (LVEF) was 39% and corresponding global longitudinal and circumferential systolic strain measurements were also significantly reduced. Trastuzumab was ceased and supportive cardiac therapy commenced. The ensuing LVEF and systolic strain measurements showed consistent improvement, so trastuzumab was recommenced (while supportive cardiac therapy continued). At this point, reduced circumferential systolic strain with preserved LVEF was observed. Subsequent echocardiograms revealed further reductions in circumferential and longitudinal systolic strain without reductions in LVEF.

7.1 PATIENT HISTORY

A 45-year-old female was referred for cardiovascular management following a recent hospital admission for heart failure. Eight months earlier she had been diagnosed with localised (lymph node negative, human epidermal growth factor receptor (HER-2/neu) positive) left-sided breast cancer. Her medical treatment for cancer had included 4 cycles of fluorouracil, adriamycin and cyclophosphamide (FAC) with a total adriamycin dose 670 mg (394mg/m2), 7 cycles of Paclitaxel (total dose 910 mg) and 2 cycles of 3 weekly trastuzumab (total dose 1000 mg). Transthoracic echocardiogram (TTE) while in hospital had revealed mild to moderate global left ventricular (LV) systolic dysfunction. Heart failure was treated with carvedilol and frusemide. Angiotensin converting enzyme inhibitors (ACEI's) were not tolerated because of cough. Paclitaxel treatment was ceased and the trastuzumab therapy temporarily withheld.

Management over the ensuing 9 months included clinical evaluation of heart failure and serial TTE's. The initial TTE revealed moderate global LV systolic dysfunction; biplane LV ejection fraction (LVEF) by Simpson's method was 39%. At the subsequent 3 visits, clinical status and cardiac function as measured by TTE gradually improved and LVEF on the 4th visit measured 52%. Given the improved clinical status and LVEF, trastuzumab therapy was recommenced and supportive cardiac therapy (carvedilol and frusemide) was continued.

Evaluation of cardiac function was extended to include two-dimensional (2D) myocardial strain imaging to test for evidence of subclinical cardiac dysfunction. Peak global LV systolic strain in the longitudinal and circumferential planes was measured by averaging peak strain from 3 consecutive cardiac cycles from 6 segments in each plane. Images were acquired using a Vivid 7 digital ultrasound system (GE Healthcare, Norway) with a 2.5MHz variable frequency transducer and measurement of 2D speckle tracking myocardial strain was performed off-line with EchoPac version BT 10 (GE Vingmed, Norway). Retrospective analysis revealed significantly reduced strain measurements on the initial TTE (compared to published normal values (Marwick, 2009)), with a consistent increase in strain on each of the following 3 echocardiograms, in keeping with the increasing LVEF. After trastuzumab was restarted however, a marked reduction in LV systolic strain was observed, when in contrast LVEF was unchanged (see figures 7.1 A and B).

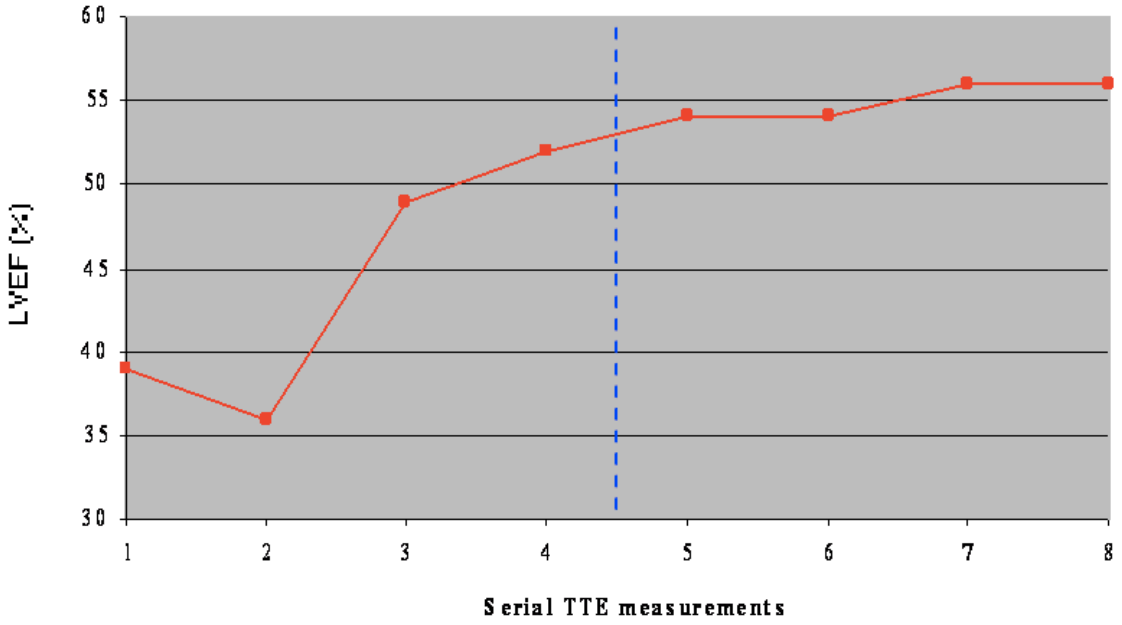

Figure 7.1 A)

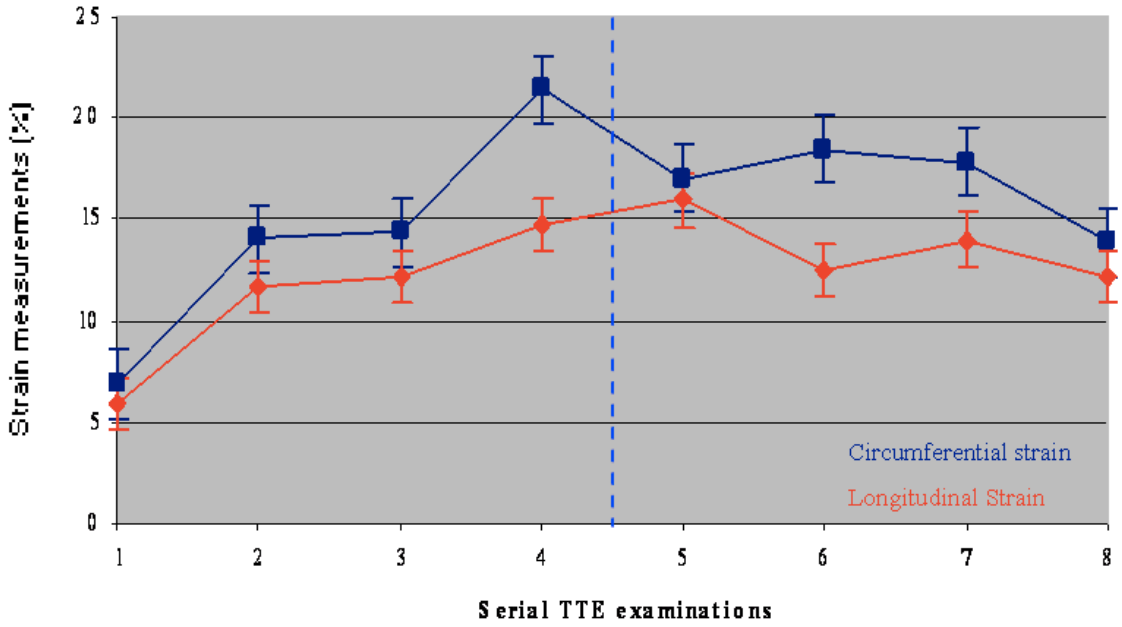

Figure 7.1 B)

Figure 7.1 A) LVEF measurements from the 8 TTE's. The vertical dotted line indicates when trastuzumab was recommenced, after which LVEF remained unchanged. B) 2D longitudinal and circumferential strain measurements from 8 TTE's. The vertical dotted line indicates when trastuzumab was recommenced, after which longitudinal and circumferential strain measurements were reduced.

Five months after recommencing trastuzumab, an exercise stress test was performed. LVEF immediately before exercise measured 56%. Heart rate peaked at 139 beats per minute (79% of the maximal age predicted heart rate; blunted response likely due to carvedilol) at maximal exercise, 9min 15sec into the Bruce protocol. Maximum blood pressure was 140/60mmHg and no evidence of myocardial ischemia was identified. Biplane LVEF (by Simpson's method) immediately after exercise measured 56%, indicating an absence of normal LV contractile reserve (at 79% of maximal age predicted heart rate). Longitudinal and circumferential strain measurements immediately after exercise were reduced when compared with those measured immediately beforehand (see images 7.2 A and B, 7.3 A and B).

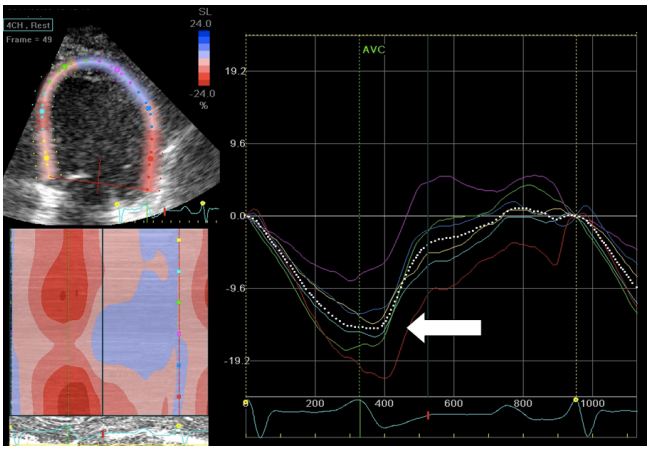

Figure 7.2 A)

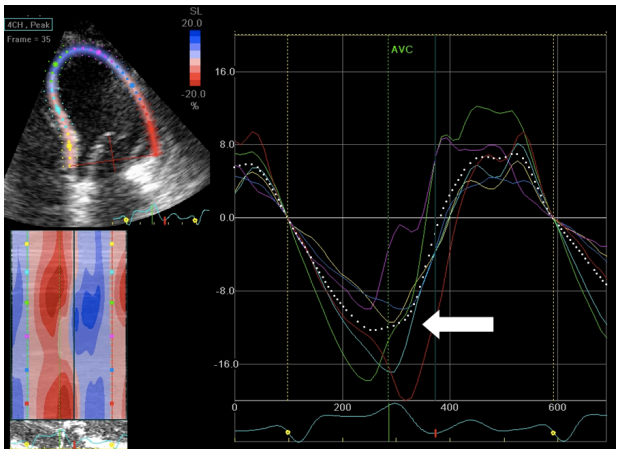

Figure 7.1 B)

Figure 7.2 2D longitudinal systolic strain measurements from the apical 4-chamber view. A) The dotted white line marks global strain. Peak longitudinal strain before exercise (indicated by the arrow) was -14.8%. B) The dotted white line marks global strain. Peak longitudinal strain after exercise (indicated by the arrow) was -12.3%.

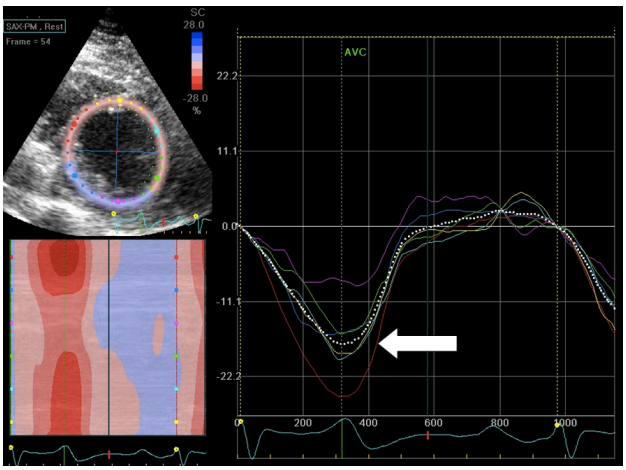

Figure 7.3 A)

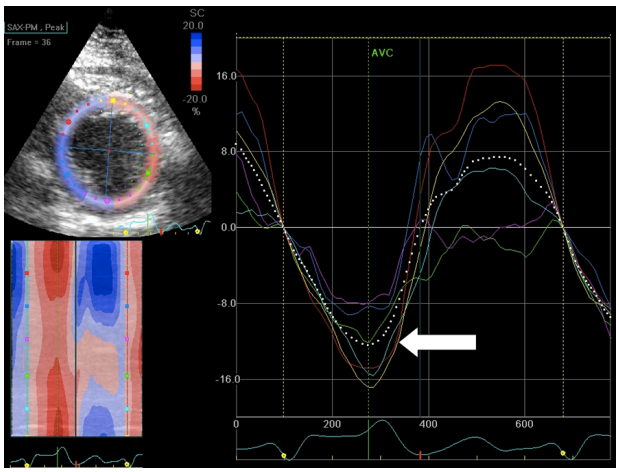

Figure 7.3 B)

Figure 7.3 2D circumferential systolic strain measurements from the parasternal short-axis view. A) The dotted white line marks global strain. Peak circumferential strain before exercise (indicated by the arrow) was -17.8%. B) The dotted white line marks global strain after exercise. Peak circumferential strain after exercise (indicated by the arrow) was - 13.9%.

7.2 DISCUSSION

LVEF is currently used to identify and monitor chemotherapy related cardiotoxicity. However, LVEF has numerous limitations, which include geometrical assumptions regarding cardiac anatomy and its inability to detect subtle changes in segmental function (Stanton, Leano, & Marwick, 2009). 2D myocardial strain imaging is a novel echocardiographic technique that may overcome many of the limitations of LVEF. Strain is a measure of myocardial deformation that enables multi-plane assessment of segmental and global LV function (Choi et al., 2009; Mizuguchi et al., 2008) and has been recommended for application in this setting (Jurcut et al., 2008b).

Recently, reduced longitudinal strain with preserved LVEF and preserved circumferential strain has been reported in both short-term (Stoodley et al., 2011) and long-term (Tsai et al., 2011) cancer survivors treated with anthracyclines. We observed reduced longitudinal and circumferential strain with preserved LVEF after the recommencement of adjuvant trastuzumab (while the patient received supportive cardiac therapy (see figures 7.1A and 7.1B)). Moreover, further reductions in longitudinal and circumferential strain were observed after an exercise stress test that also demonstrated a lack of contractile reserve by LVEF.

Unlike anthracycline cardiotoxicity, toxicity induced by trastuzumab is not dose related and is regarded as being largely reversible, although this has been called into question (Telli, Hunt, Carlson, & Guardino, 2007). The improved cardiac function observed in this case after temporary cessation of trastuzumab indicates that the cardiotoxicity was most likely due to trastuzumab and not to late anthracycline toxicity. However, the reduced strain measurements and absence of contractile reserve following trastuzumab's recommencement indicate that trastuzumab-induced cardiotoxicity may not be fully reversible and that a resting LVEF may be unable to detect these early changes in LV systolic function.

Trastuzumab therapy is life saving; however its efficacy is limited by its cardiotoxic potential, which means that close monitoring of cardiac function is necessary when trastuzumab therapy is administered. This case study supports prior publications that demonstrate strain imaging, with its unique multiplane quantification of myocardial deformation, is superior to the measurement of LV volumetric change (as measured by LVEF) in detecting reduced myocardial systolic function. It is likely that early damage involves the subendocardial fibers selectively as demonstrated by reduction in longitudinal strain with preserved LVEF. Moreover, it indicates that strain imaging may aid decision making regarding the reintroduction of trastuzumab therapy in the setting of chemotherapy induced cardiotoxicity.

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CHAPTER 8

SUMMARY, CONCLUSIONS AND FUTURE WORK

8.1 SUMMARY

The rationale for undertaking the research was addressed in the opening chapter. In brief, an enhanced understanding of tumor biology has helped to improve breast cancer treatment, and with current treatment regimens, the cancer prognosis for many breast cancer patients is very favorable. Anthracycline chemotherapy remains the cornerstone of breast cancer treatment four decades after its development. Anthracyclines possess potent anti-tumor properties, and their benefits are confirmed by a considerable body of evidence. However, their efficacy is undermined by dose dependent cardiotoxicity that mandates close monitoring of cardiac function: the method of choice for monitoring cardiac function is the measurement of left ventricular ejection fraction (LVEF) by transthoracic echocardiography. A new echocardiographic technique, myocardial strain imaging, may provide a more sensitive measurement of LV function than LVEF and improve measures aimed at countering cardiotoxicity. The aim of this research was to investigate strain imaging by comparison with LVEF, in breast cancer patients treated with anthracyclines.

Certain key aspects of echocardiography were described in chapter 2. These included descriptions of the significant technical advances during its development that have helped to achieve its current status. Echocardiography continues to develop, and an important limitation in its current practice was also described. That is, the assessment of left ventricular (LV) systolic function has always been a principal indication for echocardiography, although assessment of LV systolic function is not straightforward and its accurate and reproducible measurement remains technically challenging. Currently, resting two-dimensional (2D) LV ejection fraction (LVEF) is the key echocardiographic parameter used to quantify LV systolic function. While three-dimensional echocardiography (3DE) has been shown to be a more accurate method than 2D for measuring LVEF, neither method is well suited to evaluating myocardial function: the left ventricle has considerable functional reserve, and significant damage may occur before a reduction in LVEF.

The recent development of a novel echocardiographic technique, myocardial strain imaging, which may provide more sensitive, accurate and reproducible measurements of LV systolic function, was introduced in chapter 3. Additional details related to anthracyclines, prior research in the area of echocardiographic measurement of cardiac function in breast cancer patients and the use of strain imaging in other disease settings were also described. It is anticipated that strain imaging may enable the more timely introduction of measures aimed at countering cardiotoxicity.

Initially, fifty-two women with histologically confirmed breast cancer were prospectively studied. In chapter 4, the findings from LVEF and strain measurements made 1 week before and 1 week after anthracycline chemotherapy were presented. Reduced systolic strain immediately after treatment, indicative of early impairment of myocardial function (before detectable changes in LVEF) was observed. That is, global longitudinal LV systolic strain was reduced from -17.7 to -16.3% ($p < 0.01$) with 48% of measurements reduced by > 10%, in the absence of any reduction in LVEF > 10%.

The next chapter related the analysis of diastolic function, made 1 week before and 1 week after anthracycline chemotherapy from the same group of participants as the initial measurements of systolic function. Conventional Doppler, tissue Doppler and myocardial strain imaging were used to measure diastolic function and revealed altered LV diastolic function 1 week after anthracycline chemotherapy. Moreover, the analysis indicated that changes in diastolic function are associated with reduced systolic function - reduced early diastolic strain rate (E-Sr) being most strongly associated with reduced post chemotherapy systolic strain.

In chapter 6, the measurements of systolic function made over 12 months were presented. Her2/neu positive patients who proceeded to receive trastuzumab therapy after anthracycline treatment were excluded from the 6 and 12 month analysis (to ensure that any cardiotoxicity related to trastuzumab was excluded). Once more, LV systolic dysfunction was detected with strain imaging within 7 days of completion of chemotherapy (without a similar discernible change in LVEF). Moreover, systolic function remained reduced 6 months after treatment, and the reduced systolic strain 7 days after and 6 months after treatment occurred non-uniformly within the LV. In the majority of participants, LV dysfunction was noted to be transient, with strain values returning to normal by 12 months. In the 16% of participants with persistently reduced strain 12 months after anthracyclines, significantly higher cumulative anthracycline doses had been administered.

In brief, observations of the short and intermediate term effects of anthracyclines showed that:

1) Myocardial systolic dysfunction can be detected with strain imaging within 7 days of completing anthracyclines without a similar discernible change in LVEF.

2) Alterations in diastolic function occur immediately after anthracyclines and are related to changes in systolic function.

3) Dysfunction occurs non-uniformly within the LV (with relative sparing of the apical segments).

4) In the majority of patients, low dose anthracycline-induced myocardial dysfunction is transient, without permanent dysfunction.

5) Persistently reduced global strain occurs in ~ 16% of HER2/neu negative patients when systolic strain is reduced to a value below -17.2% at 6 months following anthracycline treatment; this persistent reduction is associated with higher cumulative anthracycline doses.

In chapter 7, the case study of a young female breast cancer patient who developed heart failure during trastuzumab therapy (following anthracyclines) was presented. Trastuzumab was ceased and supportive cardiac therapy started when the LVEF measured 39% (systolic strain measurements were also significantly low at this point). Ensuing LVEF and systolic strain measurements showed consistent improvement on therapy, so trastuzumab was recommenced (while supportive therapy continued). Subsequent echocardiograms revealed reductions in systolic strain measurements prior to changes in LVEF. An absence of contractile reserve, as measured by LVEF, was eventually noted after exercise (with further reduction in strain). This case study, while limited by an absence of pre therapy data, indicates that strain imaging may aid decision making about the reintroduction of trastuzumab after significant LV dysfunction.

8.2 CONCLUSIONS

At the outset of the research, the primary aim was to evaluate cardiac function in breast cancer patients treated with potentially cardiotoxic chemotherapy. Specifically, the current echocardiographic method, LVEF, and a contemporary method, myocardial strain imaging, were compared in order to determine which of the two techniques is better suited to monitoring potential anthracycline-induced cardiotoxicity. The five most salient observations from the research are listed above: these 5 observations enable the following conclusions to be made.

Conclusions from observations 1 and 2: To date, studies that have reported LV dysfunction immediately after anthracyclines chemotherapy have used different methods of analysis (such as the older tissue Doppler method for calculating strain). We observed reduced LV systolic and diastolic function with the 2D strain imaging technique, prior to discernible change in LVEF, within 7 days of completion of anthracyclines. To our knowledge, our study is the first to report that altered diastolic strain measurements are associated with systolic dysfunction. Moreover, while the incidence of heart failure related to anthracyclines occurs most often with higher cumulative doses, microscopic analysis reveals that myocardial damage occurs with doses of doxorubicin as low as 180 mg/m^2 (Friedman, Bozdech, Billingham, & Rider, 1978). The observations of reduced systolic and diastolic function reported in this thesis indicate that strain imaging technology is more sensitive to anthracycline-induced myocardial dysfunction than LVEF, and therefore more likely to detect early signs of myocardial damage.

Conclusions from observation 3: At baseline we observed the characteristic segmental increase in myocardial systolic strain values from base to apex in the longitudinal axis. However, after anthracyclines, a greater difference between basal and apical strain values was evident, indicative of a non-uniform disruption of LV myocardial systolic function. A similar selective segmental disruption of systolic function has been reported in adolescent patients treated with anthracyclines (Poterucha, Kutty, Lindquist, Li, & Eidem, 2012), in human epidermal growth factor receptor (HER2/neu) positive patients treated with trastuzumab with a variable history of anthracycline exposure (Sawaya et al., 2011), as well as in hypertrophic cardiomyopathy (Afonso et al., 2012). This research provides confirmation that dysfunction associated with anthracycline chemotherapy occurs non-uniformly within the LV that may represent a transient increase in wall stiffness, early tissue edema and reversible focal cellular damage[2], and emphasizes the value of using strain imaging in this setting (as LVEF is not well suited to measuring subtle segmental LV dysfunction).

Conclusions from observation 4: Marked anthracycline induced cardiotoxicity, indicated by a significant reduction in LVEF, is associated with rapid functional deterioration that is often difficult to treat (Jurcut et al., 2008; Singal & Iliskovic, 1998). Interestingly, significantly reduced systolic strain 7 days post, and 6 months post anthracycline chemotherapy was observed to resolve in the majority of patients by 12 months post treatment. That is, in the majority of patients, low dose anthracycline-induced myocardial injury is transient, without permanent dysfunction. What is also of note is the absence of any discernible change in LVEF at the three post chemotherapy time points, which reinforces the superior sensitivity of strain imaging.

Conclusions from observation 5: As frequently mentioned in the thesis, a high cumulative anthracycline dose is the most critical factor related to the development of cardiomyopathy and heart failure induced by anthracyclines (Singal & Iliskovic, 1998; Swain, Whaley, & Ewer, 2003). Dose-limiting strategies aimed at preventing cardiomyopathy are an important part of routine clinical practise. This study revealed persistently reduced global strain at 12 months occurs in ~ 16% of HER2/neu negative patients - a reduction that was associated with higher doses of chemotherapy. As such, the value of ensuring anthracycline doses are kept as low as possible is supported by the strain imaging measurements made in this research.

Furthermore, significantly reduced strain at 12 months was predicted by a global longitudinal strain (GLS) value below -17.2% at 6 months following anthracyclines. Risk stratification protocols for the early detection of cardiotoxicity are important given increased life expectancy following breast cancer treatment. Monitoring HER2/neu negative patients who receive anthracyclines with echocardiograms that utilize strain imaging, both at baseline and at 6 months following commencement of their treatment, may help treating physicians to identify those with subclinical cardiac dysfunction. Patients with GLS values greater than -17.2% may not need additional cardiac monitoring; those with GLS values below -17.2% may benefit from additional cardiac monitoring to ensure that any further LV dysfunction is promptly treated (see figure 8.1).

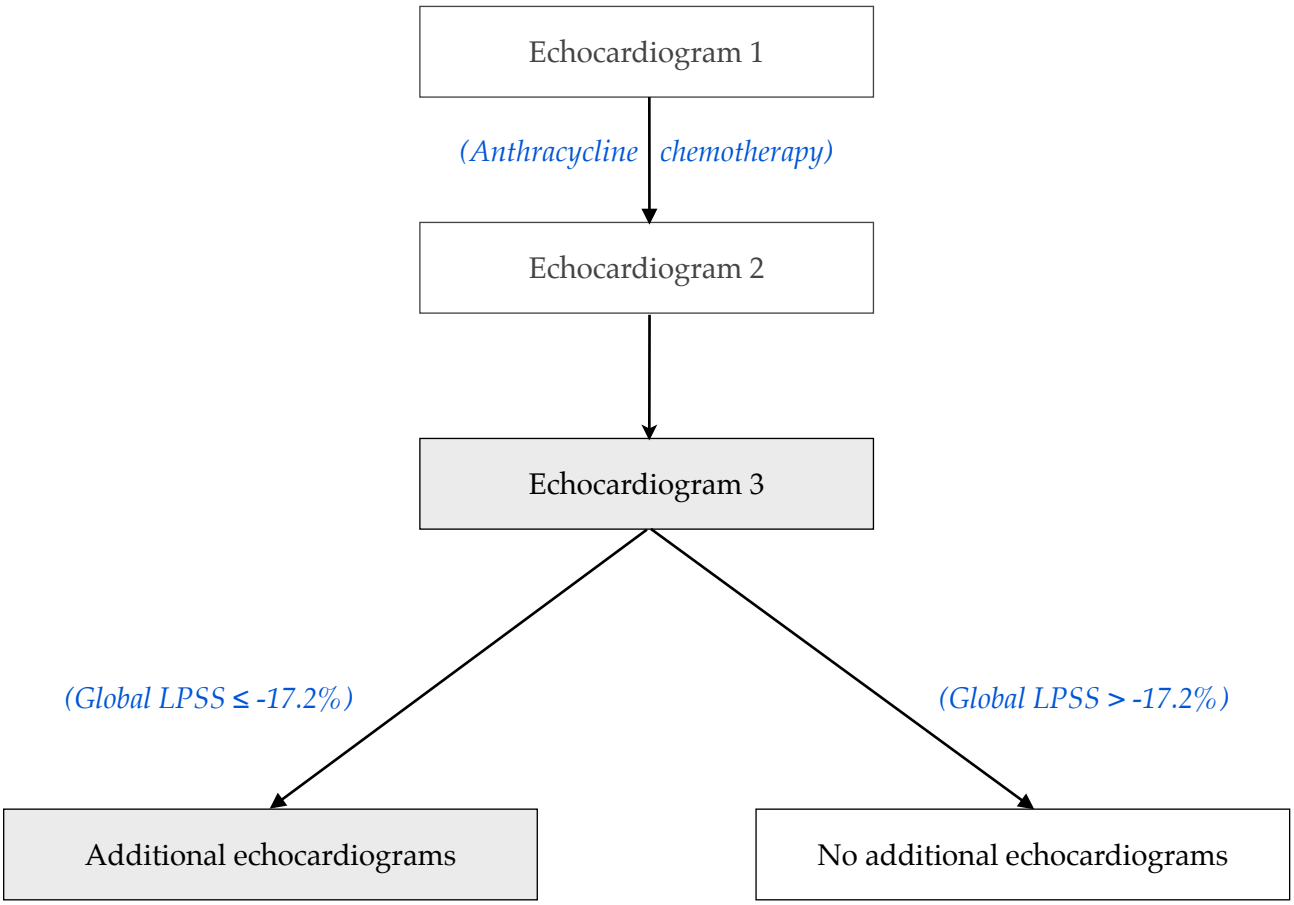

Figure 8.1. Algorithm of the monitoring protocol indicated by the results of the intermediate term follow-up of HER2/neu negative patients *(where: echocardiograms 1 and 2 are performed immediately before and immediately after anthracycline treatment, echocardiogram 3 is performed 6 months after echocardiogram 1).*

Additional observations: In keeping with previous reports, our results indicate that clinically significant reductions in LVEF (that is, LVEF reductions >10% or absolute reductions in LVEF to < 50%) following low dose anthracyclines are rare. Yet, the results also indicate that strain imaging technology is more sensitive to anthracycline-induced myocardial dysfunction than LVEF. Significantly reduced longitudinal strain after anthracycline chemotherapy has been reported in patients who later develop trastuzumab-induced cardiac dysfunction (Fallah-Rad et al., 2011), and reduced longitudinal strain after 3 months of trastuzumab therapy has been shown to predict the later development of cardiotoxicity, when LVEF was unable to do so (Sawaya et al., 2011). The majority of breast cancer patients (HER2/neu negative patients) do not require trastuzumab therapy; these are the patients we elected to focus our intermediate term study upon - which is a novel aspect of this research.

Two important conclusions can be drawn from the observations reported in chapter 7. In brief, after the reintroduction of trastuzumab, systolic strain measurements were reduced prior to changes in LVEF. An absence of contractile reserve, as measured by LVEF, was eventually noted after exercise (with further reductions in strain). Firstly, reductions in strain prior to reductions in LVEF provide further evidence that strain imaging may aid decision making about the reintroduction of trastuzumab after significant LV dysfunction. Secondly, while the toxic effect induced by anthracyclines is dose related and considered irreversible, cardiotoxicity induced by trastuzumab is not dose related and regarded as being reversible (Hayes & Picard, 2006). The absence of contractile reserve after exercise indicates that further investigation into trastuzumab's reversibility is needed.

Several echocardiographic measurements that may have enhanced the research, such as the Tei index and isovolumetric relaxation time, were not included in the study: their inclusion is anticipated in future work. Minor gender specific differences in strain were not accounted for; yet as there was only one male participant this is unlikely to have had a significant influence, especially as strain values were compared within patients (with patients serving as their own controls). Other limitations of the research are described in the discussion sections of chapters 4, 5 and 6, however two limitations are worth repeating. The first is the variability of radial systolic strain measurements (indicated by the high standard deviation) presented in chapter 4. This limitation means the results of radial strain should be interpreted with caution and is one reason why researchers are concentrating their efforts on longitudinal strain (which is far less variable). Secondly, left-sided mastectomy or lumpectomy were seen to limit strain measurements of systolic and diastolic function (due to reduced image quality) more than LVEF. While strain measurements were possible in the majority of participants, the need to use LVEF and strain in patients who have undergone left sided breast surgery is evidenced by this observation. It is an aim of the group involved in the research presented in the thesis, to further study and describe this limitation.

In conclusion, the results of this research have added to the small amount of existing data that indicates that myocardial strain imaging is a more sensitive method than the currently accepted measure of LV systolic function (LVEF) for the early detection and intermediate term monitoring of LV systolic function following anthracycline chemotherapy. Myocardial strain imaging may aid in the identification of those with preclinical LV systolic dysfunction by providing an alternative measure of myocardial function to LVEF. Our data support the need for longer-term follow-up to provide confirmation of these results, and additional evidence for a more extensive use of strain imaging in this clinical setting.

8.3 FUTURE WORK

The findings of the research presented in this thesis have led to the above mentioned conclusions, and have established a valuable knowledge base to inform future work. Yet, numerous questions arise in relation to these conclusions, and additional research is required to advance this early work. While the research indicates that myocardial strain imaging is more sensitive to early changes in myocardial function than LVEF, in the absence of significant intermediate term falls in LVEF (LVEF <50% or an absolute drop in LVEF >10%), the results of this research remain preliminary. Therefore, data which demonstrate that reduced LV function using strain imaging precedes significant drops in LVEF following anthracyclines are needed. Longer term follow-up, using the current protocol, in a larger group of participants may help to determine:

- the significance of reduced systolic and diastolic strain within 7 days of completing anthracyclines on longterm cardiovascular outcomes,

- whether the return to normal function 12 months post anthracyclines, as measured by global longitudinal systolic strain, in HER2/neu negative patients is permanent (following early reductions in strain),

- whether the addition of diastolic strain measurements 12 months post anthracyclines provides additional predictive information (such as a change in diastolic grade) for the early detection of significant LV dysfunction,

- whether myocardial function in HER2/neu negative patients with persistently reduced global LV longitudinal strain 12 months post anthracyclines deteriorates further,

- whether HER2/neu negative patients with persistently reduced global LV longitudinal strain 12 months post anthracyclines benefit from supportive therapy, and

- whether cardiovascular risk factors like diabetes, hypertension and smoking history (which were relatively low in the limited number of participants analysed to date) increase the risk of anthracycline-induced cardiotoxicity.

Answers to these questions may help to establish strain imaging cut-off values and acceptable anthracycline dosage ranges. This would enable further development of the management algorithm proposed in image 8.1, which with further development could help to identify patients for whom additional monitoring is required, and facilitate modification of chemotherapy together with the introduction of therapy to minimize the impact of cardiotoxicity.

The intermediate term observations presented in the thesis are from HER2/neu negative participants only. Including intermediate term measurements in participants found to be HER2/neu positive would be a way to build upon current findings. Due to the increased cardiotoxic potential of trastuzumab (following anthracyclines) used to treat HER2/neu positive patients, most longer term research to date has been focused on these patients. The results of the case study presented in chapter 7 of this thesis indicate a novel way of investigation. That is, adding exercise testing to the study protocol may enhance investigation of cardiotoxicity in HER2/neu positive patients.

Along with extending the duration of the research, the addition of different monitoring techniques to the protocol may further enhance the research. For example, there is growing evidence for the usefulness of blood serum biomarkers in the early identification of anthracycline-related cardiotoxicity (Dolci, Dominici, Cardinale, Sandri, & Panteghini, 2008). More specifically, early and persistent elevation of cardiac troponin (the biomarker of choice for identifying myocardial injury) has been shown to identify patients who are more likely to develop symptomatic heart failure and benefit from supportive therapies (Cardinale & Sandri, 2010). Furthermore, N-terminal pro-brain natriuretic peptide (NTpro-BNP) is associated with increased LV wall stress, and may be another useful marker for detecting anthracycline-induced cardiotoxicity (Januzzi et al., 2006; Mladosievicova, Urbanova, Radvanska, Slavkovsky, & Simkova, 2012).

In addition to including biomarker analysis, the introduction of another imaging modality would further enhance the research. Cardiac magnetic resonance imaging (CMRI), which provides highly reliable imaging of the cardiac anatomy, is the imaging modality likely to be most suitable. CMRI, when combined with late gadolinium contrast enhancement, can detect areas of irreversible myocardial damage. In a recent study of 10 patients with known LV dysfunction while receiving trastuzumab therapy, delayed gadolinium uptake indicative of myocarditis was observed in all 10 (Fallah-Rad, Lytwyn, Fang, Kirkpatrick, & Jassal, 2008). Another recent retrospective analysis of a modest number of patients with known LV dysfunction associated with trastuzumab also showed

delayed gadolinium enhancement (Wadhwa et al., 2009). For this reason, further investigation of the utility of CMRI in these patients has been recommended (Altena, Perik, van Veldhuisen, de Vries, & Gietema, 2009).

As echocardiographic imaging improves, technologies currently under development like three dimensional (3D) echocardiography strain will require closer investigation. The limitations that constrain 3D echocardiography (as outlined in chapter 2) (Picard, Popp, & Weyman, 2008), are certain to be overcome (Marwick & Narula, 2009), therefore 3D echocardiography may prove an important part of future work. For now, 2D strain imaging is significantly closer to becoming an important part of standard echocardiographic monitoring of patients treated with anthracycline chemotherapy. Future work built upon the findings presented in this thesis promise exciting discoveries and improved management of patients treated for breast cancer.

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APPENDICES

Preamble

At the commencement of the research, an application for ethics approval was made to the Sydney West Area Health Service Human Research Ethics Committee (SWAHS HREC). Following SWAHS HREC approval, ratification was requested from the University of Sydney Human Research Ethics Committee. The approval letters from both institutions are provided as appendix items 1 and 2.

As per ethics requirements, every patient recruited into the study was given an information form, and all participants provided signed consent. The original Patient Information and Consent Form approved for use in the study (version 02 dated 30/06/2008) is included as appendix item 3.

Preliminary observations from the research were submitted as abstracts to various scientific conferences at appropriate times during the course of the study. The published abstracts are included as appendix items 4 - 8.

APPENDIX ITEM 1 - SWAHS HREC APPROVAL

SYDNEY WEST AREA HEALTH SERVICE | NSW HEALTH

HUMAN RESEARCH ETHICS COMMITTEE (Westmead Campus)
Research Office, Room 2020 Clinical Sciences
Westmead Hospital, Hawkesbury Road, Westmead NSW 2145

Telephone: 02 9845 8183
Facsimile: 02 9845 8352
Email: Tina_Goodenough@wmi.usyd.edu.au

Our Ref: JH/TG HREC2008/5/4.9(2799) AU RED 08/WMEAD/125

29 July 2008

Mr Paul Stoodley
Sonographer
Westmead Private Cardiology
Westmead Private Hospital

Dear Dr Stoodley

Research Proposal: 'An investigation of echocardiographic strain imaging for improved completion screening and evaluation of left ventricular systolic function in patients receiving anthracycline chemotherapy'

Thank you for your letter dated 18 July 2008 advising the Committee regarding the number of GHPS procedures patients will undergo, however your advice is noted these scans are part of their treatment and no additional scans will be conducted for this research. Participant Information and Consent Forms Version 02 dated 30 June 2008, revised in accordance with the requests of the Human Research Ethics Committee in its letter dated 6 June 2008 are approved and a copy is attached.

As the Committee's ethical concerns have now been satisfied, **final ethical approval** of the study is confirmed for you to conduct the study at Westmead Private Cardiology, Westmead Private Hospital.

Attached please find Memorandum of Understanding signed on behalf of Sydney West Area Health Service, for your retention.

Please note the following conditions of approval:

1. The coordinating investigator will immediately report anything which might warrant review of ethical approval of the project in the specified format, including unforeseen events that might affect continued ethical acceptability of the project.
2. Proposed amendments to the research protocol or conduct of the research which may affect the ethical acceptability of the project, are provided to the HREC to review in the specific format. A copy of all proposed changes is also provided to the relevant research governance officer.

ABN: 70 667 812 600
Post Office Box 63, Penrith NSW 2751
Telephone: (02) 4734 2120 Facsimile: (02) 4734 3737

3. The HREC must be notified, giving reasons, if the project is discontinued at a site before the expected date of completion.
4. The coordinating investigator must provide an annual report to the HREC and a final report at completion of the study, in the specified format. HREC approval is valid for 12 months from the date of final approval and continuation of the HREC approval beyond the initial 12 month approval period is contingent upon submission of an annual report each year. A copy of the Annual / Final Research Report Form is attached and can be obtained electronically from the Research Office on request.
5. It should be noted that compliance with the ethical guidelines is entirely the responsibility of the researcher.

A summary of the HREC Standard Operating Procedures is attached for your reference. Should you have any queries about the HREC's Terms of Reference, Standard Operating Procedures or membership, please contact the HREC Executive Officer through the Research Office on 9845 8183 or emailing researchoffice@westgate.wh.usyd.edu.au.

In all future correspondence concerning this study, please quote your approval number HREC2008/5/4.9(2799) AU RED 08/WMEAD/125. The HREC wishes you every success in your research.

The Committee wishes you well with the study and looks forward to receiving progress reports in due course.

Yours sincerely

[redacted]

Dr Jim Hazel
Secretary
Sydney West Area Health Service
Human Research Ethics Committee

APPENDIX ITEM 2 - UNIVERSITY OF SYDNEY ETHICS RATIFICATION

The University of Sydney

NSW 2006 Australia

Human Research Ethics Committee
www.usyd.edu.au/ethics/human
Manager: Gail Briody
Telephone: (02) 9351 4811
Fax: (02) 9351 6706
Email: gbriody@usyd.edu.au
Deputy Manager: Marietta Coutinho
Telephone: (02) 9036 7568
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Human Secretariat
Telephone: (02) 9036 9308
(02) 9036 9309
(02) 9036 7274
Facsimile: (02) 9036 9310
Mailing Address:
Room 313, Level 3, Old Teachers College – A22

17 September 2008

Associate Professor David Richards
Suite 1
Westmead Private Hospital
Cnr Mons & Darcy Roads
Westmead NSW 2145

Dear Professor Richards

Title: ***An investigation of echocardiographic strain imaging for improved completion screening and evaluation of left ventricular systolic function in patients receiving anthracycline chemotherapy (Ref. No. 11308)***

Masters Student: ***Mr Paul Stoodley***

Your application was reviewed by the Executive Committee of the Human Research Ethics Committee (HREC), and in doing so has ratified your study to include the Masters student – Mr Paul Stoodley.

The Executive Committee acknowledges your right to proceed under the authority of *Sydney West Area Health Service Human Research Ethics Committee (Westmead Campus).*

Please note, this ratification has been given only in respect of the ethical content of the study.

Any modifications to the study must be approved by *Sydney West Area Health Service Human Research Ethics Committee (Westmead Campus)* before submission to the University of Sydney Human Research Ethics Committee.

Yours sincerely

[redacted]

Gail Briody
Manager
Ethics Administration

cc Mr Paul Stoodley, Suite 1, Westmead Private Cardiology, Westmead Private Hospital, Corner Mons & Darcy Roads, Westmead NSW 2145

APPENDIX 3 - INFORMATION AND CONSENT FORM

Westmead Private Hospital

PARTICIPANT INFORMATION SHEET AND CONSENT FORM

Study Title: Anthracycline Cardiac Echo Study (ACES)

Chief Investigator
A/Prof David Richards, Westmead Private Hospital

Other researchers
Mr Paul Stoodley, Sonographer, Westmead Private Hospital
A/Prof Liza Thomas, Cardiologist, Westmead Private Hospital
Dr Warwick Benson, Haematologist, Westmead Private Hospital
A/Prof Steve Meikle, Research Physicist, University of Sydney
Ms Jillian Clarke, Senior Lecturer, University of Sydney
Dr Aiden O'Loughlin, Cardiologist, Westmead Private Hospital

Invitation
You are invited to participate in a research study of new ultrasound techniques used to assess the systolic (contractile) function of the heart. Before you decide whether or not you wish to participate in this study, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully and discuss it with others if you wish.

What is the purpose of the study?
Anthracycline drugs sometimes cause the heart to contract less effectively. The purpose of the study is to investigate new ultrasound techniques that may detect reduced systolic (contractile) function of the heart at an earlier stage and more reliably than current methods.

Who will be invited to enter the study?
You are eligible to participate in this study because you have recently been diagnosed with cancer and will receive Anthracycline chemotherapy.

Do you have a choice?
Participation in this study is voluntary. It is completely up to you whether or not you participate. If you decide not to participate, it will not affect the treatment you receive now or in the future. Whatever your decision, it will not affect your relationship with the staff caring for you. New information about the treatment being studied may become available during the course of the study. You will be kept informed of any significant new findings that may affect your willingness to continue in the study. If you wish to withdraw from the study once it has started, you can do so at any time without having to give a reason. However, it may not be possible to withdraw your data from the study results if these have already had your identifying details removed.

W*estmead Private Hospital*

PARTICIPANT INFORMATION SHEET AND CONSENT FORM

Study Title: Anthracycline Cardiac Echo Study (ACES)

What will happen in the study?
If you agree to participate in this study, you will be asked to sign the Participant Consent Form. The study will be conducted over two years.

Participation will involve having up to seven cardiac ultrasound examinations (echocardiograms) over the course of the study. The first exam will occur before the commencement of chemotherapy and will take between 45 minutes and 1 hour. It will be a full study of the heart and you will be asked to answer a brief questionnaire beforehand.

The following are likely to be less than 45 minutes. They will occur on the same day, at a time as near as possible to the routine appointments and treatment that are part of the cancer management.

For every exam you will undress from the waist up and wear a hospital gown. The echocardiogram involves lying on a couch with your left arm extended on the couch. The ultrasound images are obtained using a transducer held firmly (without undue pressure) against the chest and abdomen. Three ECG dots will also be placed on the chest during the exam.

No specific preparation is required, and following the exam you are free to resume all regular daily activities.

Are there any risks?
Echocardiography (cardiac ultrasound) is a routine non-invasive imaging method. There may be mild discomfort where the transducer is applied (due to firm pressure).

Confidentiality / Privacy
Only the investigators named above whom work at Westmead Private Hospital will know whether or not you are participating in this study. Any identifiable information that is collected about you in connection with this study will remain confidential and will be disclosed only with your permission, or except as required by law. Only the researchers named above whom work at Westmead Private Hospital will have access to your details, and the results will be held securely at Westmead Private Hospital.

Compensation
If you suffer any injuries or complications as a result of this study, you should contact the study doctor as soon as possible, who will assist you in arranging appropriate medical treatment. You may have a right to take legal action to obtain compensation for any injuries or complications resulting from the study. Compensation may be available if your injury or complication is sufficiently serious and is caused by unsafe drugs or equipment, or by the negligence of one of the parties involved in the study (for example, the researcher, the hospital, or the treating doctor). If you receive compensation that includes an amount for medical expenses, you will be required to
pay for your medical treatment from those compensation monies.

Westmead Private Hospital

PARTICIPANT INFORMATION SHEET AND CONSENT FORM

Study Title: Anthracycline Cardiac Echo Study (ACES)

If you receive compensation that includes an amount for medical expenses, you will be required to pay for your medical treatment from those compensation monies. You do not give up any legal rights to compensation by participating in this study.

If you are not eligible for compensation for your injury or complication under the law, but are eligible for Medicare, then you can receive any medical treatment required for your injury or complication free of charge as a public patient in any Australian public hospital.

Will taking part in this study cost me anything, and will I be paid?
Participation in this study will not cost you anything. You will not be paid to participate.

What will happen at the conclusion of the study?
Results of the study will be provided to you, if you wish.

What happens with the results?
If you give us your permission by signing the consent document, we plan to analyze the results and publish them in peer-reviewed journals, present the results at conferences or other professional forums. The research will also be part of one of the researcher's master's degree thesis.
In any publication, information will be provided in such a way that you cannot be identified. Results of the study will be provided to you, if you wish.

Complaints
Complaints may be directed to Westmead Private Hospital Patient Representative, Ms Carmel Kennedy, telephone (02) 6687 9101 or email KennedyC@ramsayhealth.com.au, or to The Secretary, SWAHS Human Research Ethics Committee, telephone (02) 9845 8183 or email researchoffice@westgate.wh.usyd.edu.au

Contact details
When you have read this information, the researcher Paul Stoodley will discuss it with you and any queries you may have. If you would like to know more at any stage, please do not hesitate to contact him on 02 9687 0866. Alternatively, please contact A/Prof David Richards on the same number.

Thank you for taking the time to consider this study. If you wish to take part in it, please sign the attached consent form (this information sheet is for you to keep).

Westmead Private Hospital

PARTICIPANT INFORMATION SHEET AND CONSENT FORM

Study Title: Anthracycline Cardiac Echo Study (ACES)

CONSENT TO PARTICIPATE IN RESEARCH

Name of Researcher:

1. I understand that the researcher will conduct this study in a manner conforming to ethical and scientific principles set out by the National Health and Medical Research Council of Australia and the Good Clinical Research Practice Guidelines of the Therapeutic Goods Administration.
2. I acknowledge that I have read, or have had read to me the Participant Information Sheet relating to this study. I acknowledge that I understand the Participant Information Sheet. I acknowledge that the general purposes, methods, demands and possible risks and inconveniences which may occur to me during the study have been explained to me by Paul Stoodley and I, being over the age of 16 acknowledge that I understand the general purposes, methods, demands and possible risks and inconveniences which may occur during the study.
3. I acknowledge that I have been given time to consider the information and to seek other advice.
4. I acknowledge that refusal to take part in this study will not affect the usual treatment of my condition.
5. I acknowledge that I am volunteering to take part in this study and I may withdraw at any time.
6. I acknowledge that this research has been approved by the Sydney West Area Health Service Human Research Ethics Committee.
7. I acknowledge that I have received a copy of this form and the Participant Information Sheet, which I have signed.
8. I understand my identity will not be disclosed to anyone else or in publications or presentations.

Name of participant _____ Date of birth_____

Address of participant _____

Signature of participant _____ Date: _____

Name of researcher _____Date: _____

Signature of researcher _____ Date: _____

Name of witness _____ Date: _____

Signature of witness _____Date: _____

APPENDIX ITEM 4

ACUTE EFFECTS OF ANTHRACYCLINE THERAPY ON CARDIAC FUNCTION

Published in: *Heart, Lung & Circulation* 2010 Vol. 19 (supplement 2) page s162

BACKGROUND

Chemotherapy using anthracycline regimens have cardiotoxic effects. Cardiotoxicity is largely monitored using left ventricular ejection fraction (LVEF), a coarse measure of global systolic function. Myocardial strain imaging is a more sensitive tool in identification of systolic dysfunction, but to date has had limited use with respect to chemotherapy.

METHODS

20 female breast cancer patients (mean age 51 ± 9 years), with no background cardiac history, underwent an echocardiogram prior to, and within 2 weeks following chemotherapy (mean of 4.6 cycles of anthracycline). LV systolic function was evaluated by biplane LVEF and by global longitudinal 2-dimensional strain as an average from 3 beats.

RESULTS

Baseline LVEF was normal (59 ± 2%) and global longitudinal strain was -17.6 ± 2%. At follow up, a clinically insignificant reduction was noted in LVEF (post LVEF: 56 ± 3%; mean difference -3.5%); no patient had a >10% reduction in LVEF or an LVEF <50%. Paired t test between groups demonstrated a statistically significant reduction in global strain (-16.3 ± 2%; p = 0.016; mean difference -1.3%). 1 in 4 patients demonstrated a >10% reduction in strain compared to baseline value.

CONCLUSION

Although LVEF decreased, it failed to reach clinical significance immediately after chemotherapy. An overall significant reduction was noted in myocardial strain, with 20% of patients having a >10% reduction. Myocardial strain imaging may be a more sensitive measure for detecting cardiotoxicity, the early recognition of which could lead to supportive therapy.

APPENDIX ITEM 5

REDUCED SEGMENTAL LEFT VENTRICULAR SYSTOLIC STRAIN AFTER ANTHRACYCLINE CHEMOTHERAPY

Published in: *European Heart Journa*l 2011 32 (abstract supplement): page 1449

PURPOSE

The efficacy of anthracyclines is undermined by potentially life threatening cardiotoxicity. Close monitoring of cardiac function in those treated with anthracyclines is mandatory. Currently, despite numerous limitations, left ventricular ejection fraction (LVEF) by echocardiography is used for monitoring.

Myocardial strain imaging has been shown to detect left ventricular (LV) systolic dysfunction prior to noticeable changes in LVEF. Moreover, unlike LVEF, strain imaging is able to measure both segmental and global myocardial deformation. Our aim was to determine whether strain imaging would detect segmental changes in LV systolic function prior to clinically significant changes in global function as measured by LVEF.

METHODS

52 women with histologically confirmed breast cancer were prospectively studied. The first echocardiogram was performed immediately before anthracycline treatment and the second immediately afterwards. LVEF (by Simpson's method), and segmental (longitudinal, radial and circumferential) LV systolic strain were measured before and after.

RESULTS

Longitudinal systolic strain was significantly reduced (p <0.05) in 5 of 6 longitudinal segments after chemotherapy; in 3 of the 5 segments by >10%. Radial systolic strain was significantly reduced (p <0.05) in 3 of 6 regions after chemotherapy; in all 3 segments by >10%. Circumferential systolic strain was significantly reduced (p <0.05) in 1 segment only. Systolic strain was significantly reduced (p <0.05) in 9 of 18 segments (50%), 7 of 18 segments (38%) by >10%. LVEF did not fall by >10% in any patient after chemotherapy (see table).

Measurement	Observation after chemotherapy
LVEF	0 of 52 reduced by > 10%
Longitudinal strain	5/6 (83%) segments reduced (p <0.05); 3/5 reduced >10%
Radial strain	3/6 (50%) segments reduced (p <0.05); 3/3 reduced >10%
Circumferential strain	1/6 (17%) segments reduced (p <0.05)

CONCLUSION

LV systolic strain was significantly reduced in 50% of imaged segments after anthracycline chemotherapy. No reduction in global function (LVEF) >10% after chemotherapy was observed. Longer term follow up is needed to determine the clinical significance of segmental changes in LV systolic strain after chemotherapy.

APPENDIX ITEM 6

MYOCARDIAL STRAIN IMAGING DETECTS EARLY CHANGES IN GLOBAL LEFT VENTRICULAR SYSTOLIC FUNCTION AFTER ANTHRACYCLINE CHEMOTHERAPY

Published in: *European Heart Journal* 2011 32 (abstract supplement): page 4684

PURPOSE

The efficacy of anthracycline chemotherapy is undermined by potentially life threatening cardiotoxicity. Cardiotoxicity is dependent upon several factors, and its timing is variable; so close monitoring of cardiac function in those treated with anthracyclines is mandatory. Currently, despite numerous limitations, left ventricular ejection fraction (LVEF) by echocardiography is used for monitoring cardiotoxicity.

Myocardial strain imaging has been shown to detect left ventricular (LV) systolic dysfunction in several diseases prior to noticeable changes in LVEF. Our aim was to determine whether strain imaging could detect early changes in LV systolic function prior to detection by LVEF in patients after receiving anthracycline chemotherapy.

METHODS

52 women with histologically confirmed breast cancer were prospectively studied. The first echocardiogram was performed immediately before anthracycline treatment and the second immediately afterwards. LVEF (by Simpson's method), global peak longitudinal, radial and circumferential systolic strain were measured before and after.

RESULTS

Global longitudinal LV systolic strain was significantly reduced after treatment; global longitudinal strain dropped from -17.7% to -16.3% ($p < 0.01$) with 48% of global measurements reduced by >10%. Global radial LV systolic strain after treatment was also significantly reduced; global radial strain dropped from 40.5% to 34.5% ($p < 0.01$) with 59% of global measurements reduced by >10%. In contrast, no statistically significant reduction in global circumferential strain was observed (although strain was reduced >10% in 32% of participants after treatment). No reduction >10% in LVEF after chemotherapy was observed (see table).

Measurement	Before chemotherapy	After chemotherapy	% with >10% drop
LVEF (%)	58.6 ± 2.6	56.0 ± 2.8	0
Longitudinal strain (%)	-17.8 ± 2.1	16.3 ± 2.0	48
Radial strain (%)	40.5 ± 11.4	34.5 ± 11.4	59
Circumferential strain (%)	-20.3 ± 2.6	-20.0 ± 3.3	32

** $p < 0.01$ versus before chemotherapy value (Values expressed as mean ± SD)*

CONCLUSION

We observed significantly reduced global LV systolic strain early after anthracycline treatment, prior to significant reductions in LVEF. These observations may indicate early impairment of myocardial function, and warrant longer-term surveillance to determine their clinical relevance.

APPENDIX ITEM 7

ALTERED LV DIASTOLIC FUNCTION AFTER ANTHRACYCLINE CHEMOTHERAPY

Published in: *Heart, Lung & Circulation* 2011 Vol. 20 (supplement 2) page s158

BACKGROUND

Anthracycline chemotherapy is potentially cardiotoxic. Cardiotoxicity has traditionally been monitored by measuring change in left ventricular (LV) systolic function. Recent evidence suggests, however, that abnormalities of LV diastolic function can precede systolic abnormalities.

METHODS

Thirty consecutive female breast cancer patients (age: 48±10 years) with no background cardiac history were prospectively studied. Each had an echocardiogram prior to and immediately after completing chemotherapy. Twenty-five were treated with Doxorubicin (≤310 $mg.m^{-2}$) and five with Epirubicin (≤360 $mg.m^{-2}$). LV systolic function was evaluated with biplane LVEF and diastolic function with transmitral, tissue Doppler (TDI) and strain rate (SR^{-1}) measurements.

RESULTS

Average LVEF was significantly lower after chemotherapy (56±3% vs 59±3%, p <0.01). Significant changes in transmitral, TDI and SR^{-1} measures of diastolic function were also observed (see table).

Measurement	Before chemotherapy	After chemotherapy
Transmitral 'A' velocity ($m.s^{-1}$)	0.63 ± 0.13	0.67 ± 0.14*
E/E' septal	8.3 ± 2.4	9.3 ± 2.1*
E' average ($m.s^{-1}$)	9.9 ± 2.7	9.2 ± 2.8*
E/E' average	7.1 ± 2.5	8.0 ± 2.2*
ESR ($^{1/s}$)	1.03 ± 0.24	0.90 ± 0.25*

** p<0.05 vs before chemotherapy (Values expressed as mean ± SD)*

CONCLUSION

The observed reduction in LV systolic function (LVEF) after chemotherapy did not reach clinical significance. The observed reductions in multiple measures of LV diastolic function indicate an increased LV end diastolic pressure. These findings warrant further investigations in order to determine whether they are predictive of reduced LV systolic function.

APPENDIX ITEM 8

EVALUATION OF LEFT VENTRICULAR SYSTOLIC FUNCTION IN THE INTERMEDIATE TERM AFTER ANTHRACYCLINE CHEMOTHERAPY: A COMPARISON OF LVEF AND GLOBAL LONGITUDINAL STRAIN

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BACKGROUND

Anthracycline chemotherapy is potentially cardiotoxic. As toxicity has variable timing, close monitoring of those treated with anthracyclines is mandatory. Global longitudinal strain (GLS) measured by speckle tracking echocardiography can detect reduced LV systolic function before LVEF in numerous diseases. We aimed to determine, 1) whether GLS would detect reduced LV systolic function before LVEF immediately after anthracyclines and 2) whether any changes would persist at 6 and 12 months.

METHOD

Forty-one consecutive breast cancer patients were prospectively studied. The baseline echocardiogram was performed 1-week before and the 2nd 1-week after chemotherapy. The 3rd and 4th echocardiograms were performed at 6 and 12 months. GLS (measured from the apical 4 chamber view), and LVEF (by Simpson's method) were measured at each time point.

RESULTS

Repeated measures analysis showed GLS was significantly reduced 1 week after chemotherapy (-16.7 ± 4.0% from -18.3 ± 4.3%, p <0.01), although LVEF was not. Six months after chemotherapy, GLS was no longer reduced, while a clinically non-significant reduction in LVEF compared to baseline was observed (56.1 ± 3.4% from 58.0 ± 2.6%, p <0.05). Twelve months after chemotherapy, both GLS and LVEF had returned to values similar to before therapy.

CONCLUSION

Reduced GLS 1 week after anthracycline chemotherapy indicates early identification of reduced LV systolic function, undetected by LVEF, and may enable better risk assessment of patients who require additional therapies that are potentially cardiotoxic. Reductions in GLS and LVEF did not persist 12 months after chemotherapy, suggesting that these effects are transient.