Prevention of cardiac dysfunction during adjuvant breast cancer therapy

Geeta Gulati, M.D.
Department of Cardiology
Division of Medicine
Akershus University Hospital
And
Institute of Clinical Medicine
University of Oslo
Norway
# Table of Contents

1. Prologue ................................................................................................................. 4  
2. Acknowledgements ............................................................................................... 4  
3. Abbreviations ......................................................................................................... 7  
4. List of papers .......................................................................................................... 9  
5. Introduction ............................................................................................................. 10  
6. Background ........................................................................................................... 11  
   6.1. Breast cancer treatment .................................................................................. 11  
   6.2. Cardiotoxicity ................................................................................................. 11  
      6.2.1. Anthracyclines ....................................................................................... 11  
      6.2.2. Trastuzumab ......................................................................................... 13  
      6.2.3. Radiation ............................................................................................... 15  
      6.2.4. Other ..................................................................................................... 16  
   6.3. Imaging and cardiovascular biomarkers ....................................................... 16  
      6.3.1. Echocardiography ................................................................................. 17  
      6.3.2. Cardiovascular magnetic resonance ................................................... 19  
      6.3.3. Circulating cardiovascular biomarkers ............................................. 20  
   6.4. Prevention ....................................................................................................... 23  
7. The current thesis ................................................................................................... 25  
   7.1. General aims ................................................................................................... 25  
   7.2. Specific aims of the papers .......................................................................... 25  
   7.3. Material and methods .................................................................................... 26  
      7.3.1. Study design ......................................................................................... 26  
      7.3.2. Power calculation and sample size ....................................................... 27  
      7.3.3. Inclusion ............................................................................................... 28  
      7.3.4. Randomization ..................................................................................... 29  
      7.3.5. Compliance ......................................................................................... 29  
      7.3.6. Adverse events ..................................................................................... 29  
      7.3.7. CMR .................................................................................................... 30  
      7.3.8. Echocardiography .............................................................................. 30  
      7.3.9. Biomarkers ........................................................................................... 30  
      7.3.10. Statistical analysis .............................................................................. 31  
7.4. Results ............................................................................................................... 31  
   7.4.1. Inclusion ................................................................................................... 31  
   7.4.2. Randomization ......................................................................................... 32  
   7.4.3. Compliance .............................................................................................. 33
7.4.4. Adverse events ................................................................. 34
7.4.5. Summary of results for each paper .................................. 34
7.5. Methodological considerations ........................................... 38
7.6. Discussion ........................................................................ 40
7.7. Strengths and limitations .................................................... 48
7.8. Conclusions ....................................................................... 48
7.9. Clinical implications and future research ............................ 49
7.10. Closing remarks .............................................................. 49
8. References ............................................................................ 52
1. Prologue

I just wanted to do some research on the side when my supervisor introduced me to this project. At that time there was no acronym, so I can assure you the acronym “PRADA” was not the reason I accepted his offer. I told my supervisor I did not want to leave my clinical work, and he assured me, that unless I wanted to, I didn’t have to. Six years after this conversation, I can only laugh. He must have known there was no way of finishing this work without taking a break from my clinical work. I am happy he didn’t tell me at the time, because missing out on this project would have been the biggest mistake of my career.

2. Acknowledgements

No words can describe the gratitude I feel towards those who have helped me through this thesis. But, perhaps, by using the heart as an analogy, I can paint a picture of their importance. Professor Torbjørn Omland showed me the greatest honor when he asked me to become a part of his team. Without any doubt he is the main left coronary artery if this work is to be compared to the heart. If Omland had ever occluded, my whole academic career would have been over. Thankfully his ever-strongflowing advice has lifted me up and made me a strong and skillful researcher. He will always be my mentor.

My supervisor in oncology, Professor Jürgen Geisler, can be compared to the right coronary artery. He is always encouraging and has truly been the supply to my sinoatrial node making sure I need no pacemaker to keep going. Professor Anne Hansen Ree, also my supervisor, has been the parasympathetic innervation, always reading thoroughly through my work, making me laugh and relax while pointing out bits where I have been a bit hasty and not clear in my explanations. Without the parasympathetic innervation the heart will be too stressed to function properly.

The caval vein returns blood from the systematic circulation to the heart. If the caval vein is obstructed there would eventually be no further flow. The same is true for the funding of this project, if it was to be obstructed, the project could not have been completed. The main funding was by the Pink Ribbon and the Extra Foundation for Health and Rehabilitation. The University of Oslo and Akershus University Hospital supported the project financially in its final stage. AstraZeneca sponsored study medication and placebo free of charge and Abbott sponsored the reagents needed for cardiac troponin I, B-type natriuretic peptide, galectin-3 and C-reactive protein.
analyses. The inflow of participants would have been an impossible task without Dr. Berit Gravdehaug, and I am ever grateful for her enthusiasm in recruiting patients.

From the caval vein the blood flows into the right atrium. This particular part of the heart easily transfers into the staff of the Clinical Research Unit at Akershus University Hospital, especially Annika Lorentzen, Vigdis Bakkelund and Marit Holmefjord Pedersen. Their patience and optimism eased the work more than any analogy I can ever make. Also the help from the staff of the Department of Radiology, including Unni Rindén, has been invaluable in getting the patients through the claustrophobic pipe of the magnetic resonance scanner. A special thanks to Professor Jeanette Schulz-Menger in Berlin, Germany. By sharing her cardiovascular magnetic resonance (CMR) expertise, she ensured the high quality of this thesis. As part of assembling all that flowed into this project, my sincere thanks for all the help received from the Departments of Cardiology, Oncology and Department of Breast and Endocrine Surgery. Without their help in assisting and encouraging the PRADA participants and me, this study would have been hard to run.

Of my non-formal supervisors, Dr. Kjetil Steine is defiantly the right ventricle. He has been there at every step, teaching me all I know about echocardiography, listening to my complains and laughing with me when the frustrations passed. If the right ventricle is weekend, there will not be enough force to move things over to the left side and the heart will eventually collapse.

It’s amazing how easily people involved in this thesis fit into the analogy of the heart. Dr. Helge Røsjø is without doubt comparable to the left atrium. He has been the kick to get things moving. He has guided me through his own experience and is like an older brother.

The left ventricle will be all my colleagues helping me to push this thesis out to the rest of the world, there are so many names to be mentioned the list could fill this book. The same goes for all my friends (including climbing buddies) who have been so patient, supporting me in my ups and downs. A more limited number of names are those comparable to the microcirculation of the heart, because without their supply a heart failure would be inevitable. A special thanks to all the co-authors: Dr. Pavel Hoffmann, Morten W. Fagerland, Dr. Jon Norseth, Dr. Tor-Arne Hagve, Dr. Florian von Knobelsdorff-Brenkenhoff, Dr. Åse Bratland and Trygve H. Storås and the data safety and monitoring board by Dr. Pål Smith, Dr. Olav Engebråten and Fredrik Dahl.
I have deliberately left out the cardiac valves until the end. There are valves between the big vessels and the atrium and between the atrium and the ventricles. The valves let nothing pass before the pressure is high enough. There is no-one more fitting to this analogy than my co PhD student Dr. Siri Lagethon Heck. She has let nothing pass before we have thought through things thoroughly enough. If she were to ever leak or stand still there would simply be no flow. I am ever so proud we have guided each other in this PhD work, but more importantly, I cherish the moments we have spent trying to find cakes in the hospital because we had a sweet tooth. The most memorable still is celebrating highlights of the study by opening champagne bottles during working hours.

If the blood enters the heart through the caval vein, it departs through the aorta. The aorta is strong enough to not burst when the blood is pumped with all its force out of the heart. My dad Dr. Ravi Gulati, my mom Sunita Gulati and my siblings, Annie, Himanshu and Dr. Dipali Gulati have held through all my frustrations, and just as the aorta, relaxed when the worst deadlines were over. Thank you guys for always being there!

Lastly, but most importantly thank YOU, for reading this thesis. Enjoy!
3. Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2D</td>
<td>Two-dimensional</td>
</tr>
<tr>
<td>3D</td>
<td>Three-dimensional</td>
</tr>
<tr>
<td>ACE</td>
<td>Angiotensin converting enzyme</td>
</tr>
<tr>
<td>AHA</td>
<td>American Heart Association</td>
</tr>
<tr>
<td>ARB</td>
<td>Angiotensin receptor blocker</td>
</tr>
<tr>
<td>BNP</td>
<td>B-type natriuretic peptide</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CMR</td>
<td>Cardiovascular magnetic resonance</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>cTn</td>
<td>Cardiac troponin</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DSMB</td>
<td>Data safety and monitoring board</td>
</tr>
<tr>
<td>ECV</td>
<td>Extracellular volume</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>E/E'</td>
<td>Ratio of peak early (E) transmitral velocity by pulsed Doppler and peak early tissue Doppler (E') (E/E') by echocardiography</td>
</tr>
<tr>
<td>FEC</td>
<td>5-Fluorouracil, epirubicin, cyclophosphamide</td>
</tr>
<tr>
<td>FISH</td>
<td>Fluorescence in situ hybridization</td>
</tr>
<tr>
<td>GLS</td>
<td>Global longitudinal strain</td>
</tr>
<tr>
<td>HER</td>
<td>Human epidermal growth factor receptor</td>
</tr>
<tr>
<td>IQR</td>
<td>Interquartile range</td>
</tr>
<tr>
<td>LGE</td>
<td>Late gadolinium enhancement</td>
</tr>
<tr>
<td>LV</td>
<td>Left ventricular</td>
</tr>
<tr>
<td>LVEF</td>
<td>Left ventricular ejection fraction</td>
</tr>
<tr>
<td>NBCG</td>
<td>Norwegian Breast Cancer Group</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>Amino-terminal fragment of the BNP prohormone</td>
</tr>
</tbody>
</table>
OVERCOME preventiOn of left Ventricular dysfunction with Enalapril and caRvedilol in patients submitted to intensive ChemOtherapy for the treatment of Malignant hEmopathies

PRADA PRevention of cArdiac Dysfunction during Adjuvant breast cancer therapy

ProBNP Prohormone B-type natriuretic peptide

q.d Quaque die (once daily)

RCT Randomized controlled trial

ROS Reactive oxygen species

SD Standard deviation

T1 Longitudinal relaxation time
4. List of papers

Publication # I

Rationale and design of the prevention of cardiac dysfunction during an Adjuvant Breast Cancer Therapy (PRADA) Trial
Cardiology 2012;123(4):240-7 (published)

Publication # II

Prevention of cardiac dysfunction during adjuvant breast cancer therapy (PRADA): a 2 × 2 factorial, randomized, placebo-controlled, double-blind clinical trial of candesartan and metoprolol
Eur Heart J 2016 Jun 1;37(21):1671-80 (published)

Publication # III

Effect of candesartan and metoprolol on myocardial tissue composition during anthracycline treatment: the PRADA trial
Eur Heart J Cardiovasc Imagin, 2017 Jun (published)

Publication # IV

Neurohormonal Blockade and Circulating Cardiovascular Biomarkers During Anthracycline Therapy in Breast Cancer Patients: Results from The PRADA Study
J Am Heart Assoc 2017 October (in press)
5. Introduction

Breast cancer is the most common malignancy among women in Norway. In 2015 on average nine women were diagnosed with breast cancer daily\(^1\). As part of the adjuvant treatment after surgery, chemotherapy regimens with the anthracycline epirubicin are used for selected patients. About 15-20% of all breast cancer patients have an overexpression of the human epidermal growth factor receptor (HER) 2 by chromogenic in-situ hybridization or fluorescence in situ hybridization (FISH)\(^2\). HER2 overexpression is associated with a more aggressive cancer. Trastuzumab is a monoclonal antibody targeting HER2 and has been revolutionary in survival for these patients\(^3\). The overall 5-year survival for breast cancer patients in Norway is currently 89%\(^1\).

Both epirubicin and trastuzumab are known to cause left ventricular (LV) systolic dysfunction, and together they have an additive cardiotoxic effect\(^3\). Cardiotoxicity in breast cancer patients is a hazard and there is a pressing need for methods to early detect and protect the heart in these patients. This has led to a closer collaboration between cardiologists and oncologists. The awareness and emphasis of cardio-oncology has increased drastically during the time of this thesis. Figure 1 shows a steady increase of the number of PubMed citations on the search term “cardio-oncology” from 1964 to April 14, 2015\(^4\).

**Figure 1**: Number of PubMed Citations from 1964 to April 14, 2015 using the search term “Cardio-Oncology”
6. Background

6.1. Breast cancer treatment

When the PREvention of cArdiac Dysfunction during Adjuvant breast cancer therapy (PRADA) trial was designed, chemotherapy in Norway consisted of the “FEC-regimen” (5-fluorouracil, epirubicin (anthracycline) and cyclophosphamide). In addition taxanes, radiation and/or trastuzumab were initiated as recommended by the flowchart by the Norwegian Breast Cancer Group (NBCG)\(^5\). The possible treatment combinations when PRADA was designed are given in Table 1.

### Table 1: Different breast cancer treatment regimens in Norway when the PRADA protocol was drafted

<table>
<thead>
<tr>
<th>FEC 100 mg/m(^2)</th>
<th>FEC 60 mg/m(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEC 100 mg/m(^2) x 4 + trastuzumab + taxanes</td>
<td>FEC 60 mg/m(^2) x 4 + taxanes</td>
</tr>
<tr>
<td>FEC 100 mg/m(^2) x 4 + trastuzumab + taxanes + radiation</td>
<td>FEC 60 mg/m(^2) x 4 + taxanes + radiation</td>
</tr>
<tr>
<td>FEC 100 mg/m(^2) x 6 + trastuzumab</td>
<td>FEC 60 mg/m(^2) x 6</td>
</tr>
<tr>
<td>FEC 100 mg/m(^2) x 6 + trastuzumab + radiation</td>
<td>FEC 60 mg/m(^2) x 6 + radiation</td>
</tr>
</tbody>
</table>

PRADA PREvention of cArdiac Dysfunction during Adjuvant breast cancer treatment; FEC 5-fluorouracil, epirubicin, cyclophosphamide. The epirubicin dose was either 100 mg/m\(^2\) or 60 mg/m\(^2\) while 5-fluorouracil and cyclophosphamide was 600 mg/m\(^2\)

Chemotherapy combinations and dosages for adjuvant breast cancer therapy in Norway are constantly updated by NBCG. When recruitment for PRADA started in September 2011 the FEC 100 mg/m\(^2\) x 6 regimen was no longer recommended. There was another revision in September 2013. Additional immunohistochemical analyses of the tumor were recommended do decide who would benefit of postoperative chemotherapy.

6.2. Cardiotoxicity

6.2.1. Anthracyclines

Anthracyclines have long been the key element in breast cancer treatment and have since its discovery in the 1960s been associated with cardiotoxicity\(^6\). With the
increasing long-time survivorship of breast cancer patients, late onset cardiotoxicity has become a significant issue.

Briefly explained, the main mechanism of anthracycline-induced cardiotoxicity is caused by the interaction of anthracyclines with topoisomerase 2β. Topoisomerase 2β causes deoxyribonucleic acid (DNA) breakage, releasing the tension of DNA coiling. The cut is then resealed. Anthracyclines inhibit topoisomerase 2β causing DNA double strand breaks. The anthracycline/topoisomerase complex also affects the gene coding leading to mitochondriopathy and increased production of reactive oxygen species (ROS) (Figure 2). ROS induce sarcomere degeneration, mitochondrial dysfunction, DNA damage and alteration of gene expression. The summation of all the mechanisms is apoptotic and necrotic cell death.

The incidence of anthracycline-induced cardiotoxicity in contemporary breast cancer treatment is uncertain. This is because (1) the populations studied have been heterogeneous, (2) anthracycline dosages used have varied, (3) the follow-up time for examining cardiac function has varied and importantly (4) the definition of cardiotoxicity has varied. As an example, a study from 1991 used fractional shortening by motion-mode echocardiography to assess LV systolic function in long-term
survivors of pediatric malignancies treated with anthracyclines. Fractional shortening was at that time the most common way to measure global LV function and two-dimensional (2D) left ventricular ejection fraction (LVEF) measurements were not used. In this study LV systolic dysfunction was found in up to 63% of the patients followed for 10 years or longer. On the other hand, in a study from 2015 of adults with different malignancies treated with anthracyclines, the incidence of reduction in LVEF of more than 10 percentage points to a value below 50% was 9%. These patients were followed for four years. 98% of cardiotoxic events occurred within the first year and most were asymptomatic.

However, we do know that anthracycline-induced cardiotoxicity is dose-dependent and important risk factors are lifelong cumulative dosage, cardiovascular comorbidities, administering the drug as a bolus, hypertension, treatment with other cardiotoxic agents or radiation, and age >65 years.

6.2.2. Trastuzumab

15-20% of breast cancer patients are classified as HER2 positive. HER2 can be measured by immunohistochemistry on the tumor cells. The measurements are scored as 0, 1+, 2+ or 3+. Those scoring < 2+ are regarded as HER2 negative and those scoring 3+ as HER2 positive. A score of 2+ is a gray zone area and FISH is performed in addition to clarify the situation. Overexpression of HER2 indicates a more aggressive disease. If present, treatment with the monoclonal antibody trastuzumab (Herceptin) is indicated, often in addition to anthracycline-containing chemotherapy. Trastuzumab has been revolutionary in breast cancer treatment reducing the risk of recurrence with about 50%. HER2 is also expressed in cardiomyocytes, and plays an important role in cardiomyocyte survival, inhibiting apoptosis and maintaining cardiac function. When HER2 in cardiac cells are targeted by trastuzumab, the survival pathways are inhibited (Figure 3).
**Figure 3:** Cell survival through binding of neuregulin (NRG) 1 and mechanisms through human epidermal growth factor receptors (HER) and potential mechanism of trastuzumab related cardiotoxicity.

A) (1) NRG-1 binds and activates HER4, (2) HER4 then binds to HER2 (3) activating cell survival pathways involving extracellular signal-regulated kinase (ERK 1/2), phosphoinositide 3 kinase/AKT and focal adhesion kinase (FAK)/Src complex

B) (1) Trastuzumab binds to HER2 (2) and inhibits HER4 binding to HER2 (3), preventing activation of pathways signaling cell survival

In contrast to anthracycline-induced cardiotoxicity, trastuzumab-induced cardiotoxicity is not related to cumulative dose and appears to have a greater tendency to cause reversible cell dysfunction. Cardiac dysfunction occurs in 2-3% of patients receiving first-line, single-agent trastuzumab. When combined with concomitant anthracycline therapy, up to 27% may develop impaired cardiac function. Observational data raise the concern that irreversible LV systolic dysfunction may occur in up to 40% of patients treated with trastuzumab following anthracyclines. In a recent meta-analysis risk factors for developing trastuzumab-associated cardiotoxicity were previous exposure to anthracyclines, older age, arterial hypertension and diabetes.

In Norway, adjuvant trastuzumab treatment is usually initiated three weeks after completion of anthracycline-containing chemotherapy and is continued for 17 cycles with three-weekly intervals. Cardiac function is measured every third month, and a decline in LVEF of more than 10 percentage points to a value below 50% may lead to premature termination of trastuzumab treatment (Figure 4).
Figure 4: Flow chart by the Norwegian Breast Cancer Group (NBCG) on heart function monitoring during trastuzumab treatment. Left ventricular ejection fraction (LVEF) values are given in percentage.

With permission from NBCG. Translated into English

6.2.3. Radiation

Selected breast cancer patients will in addition to anthracycline-containing chemotherapy be treated with radiation. Indications for post-surgery radiation are lumpectomy, or mastectomy if the tumor altogether is ≥5 cm, if the cancer invades the skin, blood- or lymph channels, if there is a positive resection margin or with extensive lymph node involvement. The potential adverse effects of radiotherapy on the heart are damages to the microcirculation, coronary arteries and the cardiac valves. However, in recent decades radiation techniques and strategies have improved immensely. Most importantly, radiation is now planned on computed tomography scans, allowing three-dimensional (3D) based delineation of the volumes and thereby sparing of organs at risk, such as the heart. Radiation delivery to the left chest wall is done under respiratory gating, reducing the radiation exposure to the heart even further. The actual incidence of radiation-induced cardiotoxicity today is difficult to evaluate because of continuous improvements of radiotherapy technique, delay between
exposure and clinical manifestations, concomitant chemotherapy with anthracyclines, change in the treated population and failure to attribute cardiac disease to previous radiotherapy.

6.2.4. Other

The FEC regimen consists of 5-fluorouracil and cyclophosphamide in addition to the anthracycline epirubicin. Neither 5-fluorouracil nor cyclophosphamide has the same cardiotoxic profile as epirubicin. 5-fluorouracil commonly causes anginal chest pain the predominant mechanisms being vasospasm and endothelial injury. Cyclophosphamide may cause tachyarrhythmia, hypotension, myocarditis, pericardial disease and heart failure. Heart failure typically occurs within days of drug administrations and with high-dose therapy (>150 mg/kg). In contemporary breast cancer treatment lower doses of cyclophosphamide are used.

Taxanes are also a common part of the adjuvant breast cancer treatment regimen. Taxanes may reduce the elimination of anthracyclines contributing to the anthracycline-induced cardiotoxicity. Taxanes by themselves can cause bradyarrhythmias.

The cardiotoxic profile of 5-fluorouracil, cyclophosphamide and taxanes will not be discussed further in this thesis. As long-term evaluation is not part of this thesis, the side effects of endocrine therapy will not be discussed.

6.3. Imaging and cardiovascular biomarkers

Heart failure is a clinical syndrome. It is characterized by typical symptoms as breathlessness, ankle swelling and fatigue. This may be accompanied by clinical signs of pulmonary and/or systemic congestion such as elevated jugular venous pressure, pulmonary crackles and peripheral edema. Heart failure may be caused by a structural and/or functional cardiac abnormality, resulting in reduced cardiac output and/or elevated intracardiac pressures at rest or during stress. Before symptoms occur, functional abnormalities such as systolic or diastolic dysfunction are often present. Cardiotoxicity occurring as a result of breast cancer treatment is associated with systolic dysfunction that can eventually lead to heart failure. In a study of cardiomyopathies, anthracycline-induced cardiotoxicity had a particular grave prognosis with a two year mortality of about 50%. It is therefore essential to identify chemotherapy induced
cardiotoxicity early. This may be done by cardiac imaging or measuring circulating cardiovascular biomarkers.

6.3.1. Echocardiography

Echocardiography is a widely used modality to evaluate cardiac function and structure. The quality of the echocardiographic equipment and software is constantly evolving, allowing for more accurate identification of subtle changes in cardiac structure and function. Cardiac function has traditionally been measured by 2D echocardiography and expressed as LVEF. LVEF is a ratio between stroke volume and end-diastolic volume where the volumes commonly are measured in four- and two-chamber views. LV volume calculation is based on summation of a stack of elliptical disks. Important limitations are difficulties in obtaining good imaging quality visualizing the entire endocardium and avoiding apical foreshortening, as this often leads to reduced volume measurements and a false high ejection fraction. 3D echocardiography is promising as volume measurements and thus LVEF are more closely related to cardiovascular magnetic resonance (CMR) measurements (Figure 5). The intra- and inter-observer variability is also better for 3D than 2D echocardiography. However obtaining good imaging quality is still an issue.
Another novel 2D echocardiographic parameter to assess LV systolic function is peak global longitudinal strain (GLS) by speckle tracking. This technique uses the grains or speckles in the grey scale ultrasound imaging and measures their longitudinal displacement. Doing this in the four-, two- and the apical long axis views creates a bullseye plot showing myocardial shortening for 16-segments, as shown in Figure 6. An average value, referred to as the average peak GLS, is a sensitive method to detect subclinical myocardial dysfunction. An increase in GLS appears to precede the decline in LVEF[^31][^32].

Cardiotoxicity is still defined by LVEF measurements. 3D LVEF is preferred over 2D measurements because of its superior accuracy. However, worldwide the accessibility and experience with 2D echocardiography still exceeds that of 3D echocardiography and hence 2D measurements are more commonly used. There is not enough data to determine if or when change in GLS should lead to interruption or cessation of cancer treatments.

Echocardiography is still the imaging method of choice for measuring diastolic ventricular function. Diastolic dysfunction is a result of impaired LV relaxation and the
European and American societies of echocardiography recommend four parameters for identifying diastolic dysfunction. These parameters are (1) annular peak early (E) transmitral velocity by pulsed Doppler, (2) average ratio of E and peak early tissue Doppler (E’) E/E’, (3) left atrial volume and (4) peak tricuspidal regurgitation velocity. If three or more of the criteria are met, LV diastolic dysfunction is present. As a simplification, measuring E/E’ may suffice.33

Although diastolic dysfunction may precede systolic dysfunction, the predictive value of indices of diastolic dysfunction in cardio-oncology remains unproven.34

**Figure 6:** Global longitudinal strain (GLS) by speckle tracking. A. apical two-chamber view. B. apical four-chamber view. C. Apical long-axis view. D. bullseye plot showing an average longitudinal peak systolic strain of -22.5% which is normal

### 6.3.2. Cardiovascular magnetic resonance

The reference standard for quantifying cardiac volumes and hence LVEF is by CMR. CMR has high reproducibility and low variability. Its use is mostly limited due to accessibility. CMR is based on the magnetization properties of protons. Randomly scattered protons are aligned by an external magnetic field. CMR also has the benefit of providing insight into cardiac pathophysiology non-invasively by measuring the longitudinal relaxation (T1) time. In this way CMR quantifies myocardial tissue characteristics. T1 allows detecting disease in the myocyte and the interstitium without contrast. By using gadolinium contrast, areas with focal fibrosis can be enhanced and quantified. However this is less suitable for detecting diffuse fibrosis as late gadolinium enhancement (LGE) uses the difference between healthy and fibrotic myocardium.36 For detecting diffuse fibrosis extracellular volume (ECV) fraction measurements have
been promising\textsuperscript{37,38}. ECV fraction is the relationship between myocardial ECV and cellular volume. It is measured by using native (without contrast) and post-contrast T1 measurements, and hematocrit values\textsuperscript{37}.

ECV fraction = \((1 - \text{hematocrit}) \times \frac{1}{\text{post contrast T1 myocardium} - \text{1/native T1 myocardium}}\)
\[
\frac{1}{\text{post contrast T1 blood} - \text{1/native T1 blood}}
\]

ECV fraction measurement dichotomizes the myocardium into its cellular and interstitial components. Alteration in the different compartments occurs via different physiological and pathophysiological mechanisms\textsuperscript{39}. Change in ECV fraction can be due to change in the extracellular matrix or change in the total cellular volume. To differentiate between change in the extracellular matrix and the total cellular volume, total ECV can be measured. Total ECV incorporates the LV mass and is measured by ECV fraction x LV myocardial volume where LV myocardial volume = LV mass/1.05 (1.05 represents the myocardial density in g/ml)\textsuperscript{40}.

Anthracycline-induced cardiotoxicity causes diffuse rather than focal fibrosis\textsuperscript{41,42}. Sparse data exist on the utility value of ECV fraction and total ECV.

6.3.3. Circulating cardiovascular biomarkers
Contrary to imaging, measuring circulating cardiovascular biomarkers by automated assays is less investigator-dependent. Biomarkers may serve as indices of healthy or pathological processes and may reflect response to pharmacological intervention\textsuperscript{43}. Cardiac biomarkers play an increasingly important role for detecting cardiomyocyte injury (cardiac troponins) and stretch of the myocardium (B-type natriuretic peptides). Other biomarkers are less specific for the cardiovascular system but have still been associated with outcome in cardiovascular disease. For instance, C-reactive protein (CRP) is considered a marker of inflammation and galectin-3 of the development of fibrosis. Evaluation of biomarkers is less invasive and a feasible way to follow patients frequently. There is conflicting data whether circulating cardiovascular biomarkers can precede decline in LVEF\textsuperscript{44}.

Cardiac troponins (cTn)
Briefly, the cTn complex consists of cTnC, cTnI and cTnT. Of these cTnI and cTnT are genetically and immunologically distinguishable from skeletal troponin I and T.
Except for a small cytoplasmatic troponin pool, troponin is bound to the myofilament (Figure 7).45

Figure 7: Troponin complex binds to the myofilament and free troponin in the cytoplasm

High sensitivity assays for cTnI and cTnT allow for both diagnostic and prognostic evaluation in subclinical cardiovascular disease46-48. Even though cTnI and cTnT are often considered to be similar, there are important differences between the two. cTnI is a smaller molecule than cTnT, and the molecular size may influence the transfer across cell membranes. Impact of renal function, age, and sex appears to be stronger for cTnT than for cTnI. While cTnI may increase in proportion to the severity of myocardial ischemia49,50, no such association has been found with cTnT50. This suggests there are subtle differences in the etiology of cardiac injury and clearance mechanisms from the circulation. Both cTnI and cTnT have been used in cardio-oncology studies, but not compared head to head. Generally, it is believed that anthracyclines lead to an increase in circulating levels of troponin. Cardinale et al showed that persistent elevation of cTnI during high-dose anthracycline therapy is related to decline in LVEF51. However less is known of the importance of troponin increase during contemporary breast cancer treatment using high sensitivity assays.

The role of troponin during additional therapy with radiation and trastuzumab is less clear44. This may be attributed to the different cardiotoxic mechanisms of the different anti-cancer agents.
**B-type natriuretic peptide (BNP)**

Briefly, the prohormone of BNP (proBNP) is secreted by the cardiac ventricles in response to excessive stretching of the cardiomyocytes. Upon release, proBNP is split into an inactive amino-terminal fragment, (NT-proBNP), and the active BNP. (Figure 8).

**Figure 8:** prohormone B-type natriuretic peptide (proBNP) is split into inactive amino-terminal fragment (NT-proBNP) and the active BNP. \( T_{1/2} \) denotes half-life.

![Diagram of BNP cleavage](image)


BNP causes vasodilatation and natriuresis\(^{52,53}\). B-type natriuretic peptides provide strong prognostic information across the spectrum of cardiovascular disease\(^{54-56}\). The role of BNP and NT-proBNP as diagnostic and prognostic markers in cardio-oncology is unclear\(^{44}\). This may be due to different sensitivities of the assays used, different combinations and dosages of the chemotherapy given and heterogeneity and comorbidity in the population studied.

**Galectin-3**

Galectin-3 is involved in fibrosis and heart failure progression\(^{57}\). Little is known of galectin-3 release during breast cancer treatment. Putt et al measured amongst other circulating cardiovascular biomarkers galectin-3 at baseline and every third month during anthracycline, taxane and trastuzumab therapy. There was no increase in galectin-3 during this time\(^{58}\). Boxtel et al measured galectin-3 in patients one year after
completed anthracycline therapy, there were no baseline values. Abnormal galectin-3 values were found in 7.3% of the patients, there was no correlation to LVEF\textsuperscript{59}.

**CRP**

CRP is a marker of inflammation. Increased level of CRP measured by high sensitivity assays is associated with a higher risk of cardiovascular mortality and morbidity in the general population\textsuperscript{60,61}. Sparse data are available regarding the role of CRP in cardio-oncology. In a rat study a 7.28 fold increase in plasma CRP was shown two weeks after the administration of a single dose of the anthracycline Adriamycin\textsuperscript{62}. Putt et al measured amongst other circulating cardiovascular biomarkers CRP at baseline and every third month during anthracycline, taxane and trastuzumab therapy. There was a significant increase of CRP three months after initiation of anthracycline treatment\textsuperscript{58}.

**6.4. Prevention**

Different measures have been taken to prevent anthracycline-induced cardiotoxicity, such as changing the molecular compound of the drug\textsuperscript{63}, lowering peak and cumulative doses\textsuperscript{64} and administering anthracycline as a slower infusion\textsuperscript{65}. Dexrazoxane is the only approved drug to have shown a protective effect of anthracycline-induced cardiotoxicity\textsuperscript{12,66,67}. Previously the main mechanism of the cardioprotective effect of dexrazoxane was believed to be its iron chelating effect\textsuperscript{66,67}, the theory being consistent with the previous main hypothesis that iron played a central role in anthracycline-induced cardiotoxicity. Newer data suggests that the main mechanism for dexrazoxanes cardioprotective effect is depletion of topoisomerase 2β, preventing the anthracycline-topoisomerase 2β induced DNA damage\textsuperscript{68,69}. Concerns have been raised that dexrazoxane may reduce the efficacy of cancer treatment, hence it is not commonly used in breast cancer patients. The European usage of dexrazoxane is only approved for adults with advanced or metastatic breast cancer who have been treated with a cumulative doxorubicin dose of >300 mg/m\textsuperscript{2} or epirubicin dose of >540 mg/m\textsuperscript{2}, and would benefit from continued anthracycline therapy\textsuperscript{12}. Dexrazoxane is not used in Norway.

Contemporary adjuvant breast cancer treatment regimens still increase the risk of developing LV systolic dysfunction\textsuperscript{70}. Instead of directing the protective therapy to alter the cancer treatment, preventive measures using cardio protective medication are
now being attempted. Some studies have suggested that beta blockers and angiotensin antagonists may have a cardio protective effect on anthracycline-induced cardiotoxicity. However, most of these studies are small, and many lack a randomized, placebo-controlled, double-blind design. In addition, echocardiography and not CMR has been used to measure cardiac function (Table 2).\textsuperscript{71}

**Table 2:** Overview of studies using beta blockers and angiotensin antagonists to prevent anthracycline-induced cardiotoxicity

<table>
<thead>
<tr>
<th>Study</th>
<th>Intervention</th>
<th>Initiation of study medication and time of treatment</th>
<th>Malignancy (n)</th>
<th>LVEF definition and imaging modality</th>
<th>Study design</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kalay et al 2006</td>
<td>Carvedilol</td>
<td>Before chemo., maintained for 6 months</td>
<td>Breast Cancer 34 Other 16</td>
<td>LVEF&lt;50% Echocardiography</td>
<td>Single blinded Placebo controlled Randomized</td>
</tr>
<tr>
<td>Kaya et al 2013</td>
<td>Nebivolol</td>
<td>Before chemo., continued for 6 months</td>
<td>Breast Cancer 45 No definition</td>
<td>Echocardiography</td>
<td>Double blinded Placebo controlled Randomized</td>
</tr>
<tr>
<td>Cardinale et al 2006</td>
<td>Enalapril</td>
<td>After chemo., maintained for 1 year</td>
<td>Breast Cancer 29 Other 85</td>
<td>LVEF&lt;50% and &gt;10% LVEF reduction Echocardiography</td>
<td>Open labeled Randomized</td>
</tr>
<tr>
<td>Dessi et al 2013</td>
<td>Telmisartan</td>
<td>Before chemo., maintained up to 6 months after epirubicin discontinuation</td>
<td>Breast Cancer 18 Other 31</td>
<td>No definition Echocardiography</td>
<td>Single blinded Placebo controlled Randomized</td>
</tr>
</tbody>
</table>

Modified from Yun et al. Postgrad Med J, September 2015
7. The current thesis

7.1. General aims

The protocol of the PRADA study took into account the limitations of previous studies and was therefore designed as a randomized, placebo-controlled and double-blind study. To have a homogenous cancer population, only breast cancer patients were recruited. The main purpose of the study was to determine whether cardiotoxicity related to breast cancer treatment could be prevented by concomitant use of the beta blocker metoprolol succinate and the angiotensin antagonist candesartan cilexetil. CMR was chosen as the primary imaging modality as it is highly accurate and has low variability. In addition, echocardiography was performed to evaluate 3D LVEF, GLS and diastolic function. Blood was drawn to analyze the levels of circulating cardiovascular biomarkers. A statistical analysis plan with detailed primary, secondary and tertiary aims with efficacy endpoints was drafted and sealed before study completion. The study protocol was approved by the Regional Ethics Committee of South-Eastern Norway (2010/2890) and was registered in the ClinicalTrials.gov registry (NCT01434134) prior to study initiation. All participants provided written, informed consent.

7.2. Specific aims of the papers

Paper # I published in Cardiology Vol 123 in 2012
This was a clinical trial design paper describing the rationale and design of the PRADA study.

Paper # II published in European Heart Journal volume 37 in 2016
The aim of this paper was to test the primary hypothesis that concomitant therapy with the angiotensin receptor blocker (ARB) candesartan cilexetil and/or the beta blocker metoprolol succinate would alleviate the decline in LVEF associated with contemporary adjuvant breast cancer therapy. LVEF was measured by CMR.

Paper # III published in European Heart Journal Cardiovascular Imaging, 2017
This paper highlights myocardial tissue composition as assessed by CMR during anthracycline treatment. The aim of this paper was to test the hypothesis that contemporary anthracycline treatment for adjuvant breast cancer is dose-dependently
associated with increased myocardial ECV fraction and total ECV, as well as reduced total myocardial cellular volume and that these changes could be prevented by concomitant use of candesartan and/or metoprolol.

*Paper # IV in press in Journal of American the American Heart Association, 2017*

This paper highlights the use of circulating cardiovascular biomarkers during contemporary anthracycline treatment for adjuvant breast cancer therapy. The objectives of this paper were to (1) longitudinally assess change in circulating cardiovascular biomarkers reflecting myocardial injury (cTnI and cTnT), dysfunction (NT-proBNP and BNP), inflammation (CRP) and fibrosis (galectin-3), (2) evaluate the effect of neurohormonal blockade with candesartan and/or metoprolol on the biomarker response and (3) evaluate associations between changes in biomarker concentrations and subsequent LV dysfunction in patients with early breast cancer.

**7.3. Material and methods**

**7.3.1. Study design**

The PRADA study was designed as a randomized, 2x2 factorial, placebo-controlled, double-blind trial.

End-of-study was defined as end of therapy. End of therapy differed with the different anti-cancer treatment regimens (Figure 9).
**Figure 9:** End-of-study for the different anti-cancer treatment regimens. In all 5-fluorouracil, epirubicin, cyclophosphamide (FEC) regimens, the dosage of 5-fluorouracil and cyclophosphamide was 600 mg/m². For epirubicin the dosage was 60 mg/m² (FEC 60) or 100 mg/m² (FEC 100). The FEC regimen was either given as four (FEC x 4) or six (FEC x 6) cycles with three-weekly intervals. EOS denotes end-of-study, w denotes weeks.

---

7.3.2. **Power calculation and sample size**

The power calculations were based on the following: if baseline LVEF is 60% (±5% standard deviation (SD))) and with an alpha of 0.05, and power (1-beta) of 0.95, 26 patients treated with candesartan and 26 patients treated with metoprolol would be required to detect an absolute LVEF difference of 5 ±5% (SD) percentage points between the placebo and metoprolol group and candesartan group. No interim analysis was planned and, accordingly, there were no modification of p-value thresholds or statistical stopping rule.
7.3.3. *Inclusion*

Patients with early breast cancer who had undergone surgery were recruited prospectively at the surgical outpatient-clinic at Akershus University Hospital, Lørenskog, Norway. If adjuvant chemotherapy with epirubicin was indicated, patients were recruited according to predefined inclusion and exclusion criteria as shown in Table 3.

**Table 3: Inclusion and exclusion criteria for the PRADA study**

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Woman</td>
<td>Hypotension, defined as systolic blood pressure &lt; 110 mmHg</td>
</tr>
<tr>
<td>Age 18-70 years</td>
<td>Bradycardia, defined as heart rate &lt; 50 beats/minute</td>
</tr>
<tr>
<td>Eastern Cooperative Oncology Group performance</td>
<td>Prior anthracycline chemotherapy regimen</td>
</tr>
<tr>
<td>status 0–1</td>
<td>Prior malignancy requiring chemotherapy or radiotherapy</td>
</tr>
<tr>
<td>Serum creatinine &lt; 140 μmol/L or estimated</td>
<td>Symptomatic heart failure</td>
</tr>
<tr>
<td>creatinine clearance &gt; 60 ml/min (using the</td>
<td>Systolic dysfunction (LVEF &lt; 50%)</td>
</tr>
<tr>
<td>modification of diet and renal disease formula)</td>
<td>Clinically significant coronary artery disease, valvular heart disease, significant arrhythmias or conduction delays.</td>
</tr>
<tr>
<td>Systolic blood pressure &gt; 110 mgHg and &lt; 170</td>
<td>Uncontrolled arterial hypertension defined as systolic blood pressure &gt; 170 mmHg</td>
</tr>
<tr>
<td>mmHg and LVEF &gt; 50%</td>
<td>Treatment with ACEI, ARB or beta blocker within the last 4 weeks prior to study start</td>
</tr>
<tr>
<td></td>
<td>Intolerance to ACEI, ARB or beta blocker</td>
</tr>
<tr>
<td></td>
<td>Uncontrolled concomitant serious illness</td>
</tr>
<tr>
<td></td>
<td>Pregnancy or breastfeeding</td>
</tr>
<tr>
<td></td>
<td>Active abuse of drugs or alcohol</td>
</tr>
<tr>
<td></td>
<td>Suspected poor compliance</td>
</tr>
<tr>
<td></td>
<td>Inability to tolerate the CMR scanning protocol</td>
</tr>
</tbody>
</table>

LVEF: left ventricular ejection fraction, ACEI: angiotensin converting enzyme inhibitor, ARB: angiotensin receptor blocker, CMR: cardiovascular magnetic resonance

In general all adjuvant chemotherapy and trastuzumab treatment was given at the Department of Oncology, Akershus University Hospital. During the summer holidays, end of June to beginning of August, a minority of patients received trastuzumab infusion at other hospitals in Norway. None of the patients were treated outside of Norway.

As Akershus University Hospital is not yet a radiotherapy center, adjuvant radiation was in general given at Oslo University Hospital.
has two radiotherapy centers; Ullevål University Hospital and the Norwegian Radium Hospital. Only Ullevål University Hospital had respiratory-gated radiation during the PRADA-study and all patients with left sided breast cancer and radiation indication were referred there. Hypofractionation was introduced in June 2015, hence from then radiation treatment changed fractionation from 2Gy x 20 to 2.67 Gy x 15. Due to capacity problems at Oslo University Hospital some patients received radiotherapy at Innlandet Hospital, Gjøvik.

7.3.4. Randomization

Participants were randomized in a 1:1:1:1 fashion. Treatment combination consisted of: candesartan cilexetil 32 mg q.d. and metoprolol succinate 100 mg once daily (q.d.); candesartan 32 mg q.d. and placebo q.d.; metoprolol 100 mg q.d. and placebo q.d.; or placebo and placebo q.d. A study statistician created the randomization list by using a permuted block randomization with undisclosed and variable blocking factor 4:8. Patients were stratified according to planned trastuzumab therapy. Sealed, opaque envelopes with treatment codes were stored in a locked cabinet in the offices of the Department of Clinical Research, Division of Medicine and at the Pharmacy at Akershus University Hospital. The randomization sequence was concealed from investigators and participants. Study medication and matching placebos were delivered free of charge by AstraZeneca. For each intervention tablet, the matching placebo was identical in appearance. Each study drug box contained 100 tablets. The boxes were labeled and stored at the Pharmacy at Akershus University Hospital.

7.3.5. Compliance

Compliance was registered by counting residual tablets at every second visit during FEC treatment and every third visit during trastuzumab treatment. This was compared to medication diary the patients were asked to keep.

7.3.6. Adverse events

As both metoprolol and candesartan have been on the market for a long time and are generally well tolerated, the Data Safety and Monitoring Board (DSMB) predefined what should not be reported as adverse events. This was mainly events that were thought related to anticancer treatment as hypotension, bradycardia, gastrointestinal-
symptoms, fatigue, concentration problems, insomnia, dry cough, serum creatinine increase up to 1.7 mg/dL, hyperkalemia up to 5.5 mmol/L, neutropenic fever and hair loss. If hospital admissions due to hypotension or bradycardia occurred, it was to be reported to the DSMB but not to the Norwegian Medicines Agency. Unblinding was deemed necessary if second or third degree atrioventricular block occurred, serum creatinine increased above 1.7 mg/dL or potassium increased above 5.5 mmol/L.

7.3.7. CMR
All CMR examinations were performed on the same 1.5-T CMR scanner (Achieva; Philips Medical Systems, Best, The Netherlands), using a five-element phased-array cardiac coil. Native and post-contrast T1 maps were generated by dedicated, commercially available software (cmr42, version 5.2.0, Circle Cardiovascular Inc. Calgary, Canada). LGE images were acquired 10 minutes after intravenous injection of 0.2 mmol/kg Gadolinium-DOTA (Dotarem®, Guerbet, France). All image analyses were performed by a single, board-certified radiologist blinded for treatment allocation and study order. To measure ECV, hematocrit measurements were necessary. The decision to measure ECV was decided after study initiation September 2011. Hence hematocrit was not included in the blood samples before March 2012.

7.3.8. Echocardiography
Transthoracic echocardiography was performed using a Vivid E9 (GE Vingmed, Horten, Norway). Images were digitally stored for offline analyses on custom software (EchoPAC, GE Vingmed, Horten, Norway). All image analyses were performed by a single, board-certified physician blinded for treatment allocation and study order.

7.3.9. Biomarkers
Non-fasting samples of venous blood were drawn. Serum tubes were stored at room temperature for clot formation to occur while heparin tubes were kept on ice and ethylenediaminetetraacetic acid (EDTA) tubes in room temperature. All tubes were processed within 60 minutes with centrifugation at 4000 ramp per minutes for 15 minutes and stored at -80 °C.

Before analyses thawed specimens were mixed thoroughly by low-speed vortexing until visible homogeneity. EDTA-plasma specimens were centrifuged at
13 500 and serum specimens at 3500 relative centrifugal force for 30 minutes, the clear supernatants were then transferred to the sample cups. Abbott Diagnostics delivered reagents free of charge to measure serum cTnI, plasma BNP, plasma galectin-3 and serum CRP. The cTnI, BNP and galectin-3 analyses were performed by the Section for Medical Biochemistry, Division for Diagnostics and Technology, Akershus University Hospital. The CRP analysis was performed at Clinic for Medical Diagnostics, Vestre Viken Hospital Trust, Drammen, which is 34 km from Akershus University Hospital. The samples were put on dry ice and delivered by car to Vestre Viken Hospital. Serum cTnT and serum NT-proBNP analyses were performed by Section for Medical Biochemistry, Division for Diagnostics and Technology at Akershus University Hospital. High sensitivity assays were used to measure cTnI, cTnT and CRP.

7.3.10. Statistical analysis
A statistical analysis plan was signed and sealed prospectively. For the primary results a linear mixed model was used. All analyses were done according to the intention-to-treat principle unless otherwise stated. Normally distributed continuous data are all presented as mean ± SD, non-normally distributed continuous data as median and interquartile range (IQR), and categorical variables as proportions. For normally distributed continuous data paired-sample and independent sample Student’s t-tests were used to assess within and between group differences, for non-normally distributed continuous data Wilcoxon Signed Rank and Mann-Whitney U tests were used. We did not impute any of the missing data.

7.4. Results
7.4.1. Inclusion
The study took longer time than originally anticipated. Study inclusion was planned to start in spring 2011 and be completed by December 2013. However, the inclusion did not commence before autumn 2011 as (1) approval from the Regional Committees for Medical and Health Research Ethics was delayed, (2) due to practical reasons, such as avoiding recruitment start during the summer holidays, and (3) due to logistical reasons as capacity issues at the CMR laboratory. The NBCG updated their treatment protocol recommendations in September 2009 and September 2013, resulting in fewer eligible participants for the PRADA trial than originally anticipated.
In February 2014 the steering committee of the PRADA study took steps as not to delay the study more than necessary. 111 women were randomized by February 2014, 29 (26%) of these were HER2 positive as measured by immunohistochemistry or in situ hybridization. HER2 positive patients are in treatment for about 60 weeks. At this time point the last HER2 positive patient was included in January 2014 and would be in treatment until February/March 2015. HER2 negative patients are in treatment for up to 30 weeks. If the last HER2 negative patient was to be recruited in September 2014, she would complete her treatment around the same time as the last HER2 positive patient from January 2014. This selection was deemed necessary as firstly the durability of the study medication from AstraZeneca was until March 2015, secondly the initial funding for the PhD work ceased by spring 2014 and current funding was temporarily done by the Department of Research, Akershus University Hospital. Lastly there was the consideration that PRADA was targeting an important hypothesis in the field of cardio-oncology and that a delay in publication would reduce the novelty of the study. By this time the proportion of HER2 positive patients recruited were higher than anticipated. Reaching the target of 120 women by September 2014 was considered achievable, a maximum limit of 150 women was set. Hence it was enacted that recruitment would continue until September 30, 2014 and 130 patients were randomized in the PRADA-study.

7.4.2. Randomization

130 women were randomized and four of these were excluded due to randomization failure including change in treatment plan, later acknowledgement of previous radiation treatment to the chest and cardiovascular complication prerandomization. Hence the PRADA study consisted of 126 validly randomized participants (Figure 10).

Paper # II:
The primary analyses were based on the change in LVEF as assessed by CMR. Of the 126 participants additionally six were excluded due to missing CMR pre- or post-breast cancer treatment. 120 participants constitute the population of this paper (Figure 10)

Paper # III:
This paper focuses on novel non-invasive methods of CMR to assess and quantify myocardial tissue composition. This required measuring hematocrit. Hematocrit was
included in the blood sampling package from March 2012. Of the 126 validly randomized women additionally 57 were excluded due to missing hematocrit measurements, ECV measurements or missing CMR pre- or post- anthracycline treatment. 69 participants constitute the population of this paper (Figure 10).

Paper # IV:
This is a paper on circulating cardiovascular biomarkers. Of the 126 validly randomized women, additional five were excluded due to incomplete anthracycline therapy or missing post-anthracycline blood samples. 121 participants constitute the population of this paper (Figure 10).

![Figure 10: CONSORT diagram of patients included in the different papers](image)

7.4.3. Compliance
Compliance with study drugs was generally excellent. For the primary results, two patients did not adhere to the assigned candesartan, one to the candesartan placebo, three to metoprolol and three to the metoprolol placebo. The mean daily study drug
dose was 23 ± 11 mg for candesartan, 26 ± 9 mg for candesartan placebo, 68 ± 34 mg for metoprolol and 78 ± 32 mg for metoprolol placebo from initiation to end of intervention.

7.4.4. Adverse events

In total nine serious adverse events occurred. This is shown in Table 4. No patient in the intention-to-treat analysis withdrew because of adverse events.

<table>
<thead>
<tr>
<th>Treatment Arm</th>
<th>Number of Serious Adverse Events</th>
<th>Type of Serious Adverse Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candesartan-metoprolol</td>
<td>2</td>
<td>Thrombus in right atrium, vasovagal syncope</td>
</tr>
<tr>
<td>Candesartan-placebo</td>
<td>2</td>
<td>Loss of muscle tonus/epilepsy, deep venous thrombosis</td>
</tr>
<tr>
<td>Metoprolol-placebo</td>
<td>4</td>
<td>Rash (1 week before end of study), nasopharyngitis, thrombophlebitis, pneumonia</td>
</tr>
<tr>
<td>Placebo-placebo</td>
<td>1</td>
<td>Depression</td>
</tr>
</tbody>
</table>

7.4.5. Summary of results for each paper

Paper # I

**Rationale and design of the prevention of cardiac dysfunction during an Adjuvant Breast Cancer Therapy (PRADA) Trial**

This is a paper of the methodology of the PRADA-study, hence no results are presented.

Paper # II

**Prevention of cardiac dysfunction during adjuvant breast cancer therapy (PRADA): a 2x2 factorial, randomized, placebo-controlled, double-blind clinical trial of candesartan and metoprolol**

By including 130 patients with early breast cancer, PRADA was the largest preventive randomized, 2 x 2 factorial, placebo-controlled, double-blind clinical trial with candesartan, metoprolol and matching placebos published by February 2016. The primary outcome was change in LVEF by CMR from baseline to completion of breast cancer treatment. The overall decline in LVEF from baseline to the end-of-study was 2.6 [95% confidence interval (CI) 1.5, 3.8] percentage points in those not assigned to
candesartan and 0.8 [0.4, 1.9] percentage points in those assigned to candesartan in the intention-to-treat analysis (p for between-group-difference in linear mixed model analysis: p=0.026). The effect of candesartan on LVEF was consistent across predefined subgroups with no significant interaction when stratified according to age, current smoking, history of hypertension, body mass index, trastuzumab or radiation (Figure 11)72. The overall decline in LVEF in those not assigned to metoprolol was 1.8 percentage points [0.7, 3.0] and 1.6 percentage points [95% CI 0.4, 2.8] in those assigned to metoprolol (between group p-value 0.772). Hence candesartan attenuated the decline in LVEF during contemporary early breast cancer treatment, while metoprolol did not.
Figure 11: Changes in LVEF is expressed in percentage points with 95% confidence intervals (CI). Concomitant therapy with candesartan alleviated the decline in LVEF observed in the placebo group. This effect was consistent across subgroups with no formal interaction observed when patients were stratified according to age, current smoking, history of hypertension, body mass index, trastuzumab or radiation. EOS, End-Of-Study; LVEF, Left Ventricular Ejection Fraction by Magnetic Resonance Imaging; BMI, Body Mass Index. Median age at baseline was 49 years, median BMI at baseline was 25.6 kg/m²

![Diagram showing the effect of candesartan treatment on differences in change in LVEF (95% CI) from baseline to EOS.](image)

Gulati et al. Eur Heart J. 2016. Reprinted with permission from Oxford University Press and Copyright Clearance Center

Paper # III

Effect of Candesartan and Metoprolol on Myocardial Tissue Composition during Anthracycline Treatment: The PRADA-trial

In this paper we studied if there were subclinical structural myocardial changes during anthracycline-containing chemotherapy and if these were affected by concomitant use of candesartan and/or metoprolol. 69 of the 130 participants had validly measurable ECV fraction, total ECV and total cellular volume pre- and post- anthracycline treatment. Those treated with a cumulative epirubicin dose of 400 mg/m² were...
associated with greater increase in ECV fraction than those treated with a cumulative epirubicin dose <400 mg/m² (between group difference 2.6 (95% CI 0.8, 4.5) percentage points, p=0.006). Higher epirubicin dose was also associated with a greater increase in total ECV (between group difference 1.8 (0.1, 3.6) mL, p=0.040) and a greater decline in LVEF (between group difference 4.1 (1.2, 6.9) percentage points, p=0.006) than lower epirubicin doses. Those assigned to candesartan had a reduction in total cellular volume compared to those not assigned to candesartan (between group difference 2.9 (1.0, 4.8) mL, p=0.003). Candesartan also attenuated the decline in LVEF (between group difference -2.7 (-4.8, -0.5) percentage points, p=0.015) compared to those not assigned to candesartan. No effect on total cellular volume or LVEF was seen on those assigned to metoprolol compared to those not assigned to metoprolol. Hence the salient finding of this study was that anthracycline therapy was associated with dose-dependent increase in ECV fraction and total ECV. Concomitant treatment with candesartan, but not metoprolol reduced LV total cellular volume.

Paper # IV

**Neurohormonal Blockade and Circulating Cardiovascular Biomarkers During Anthracycline Therapy in Breast Cancer Patients: Results from The PRADA Study**

In 121 patients, blood samples were obtained to measure change in circulating cardiovascular biomarkers for cardiac injury (cTnI, cTnT), dysfunction (BNP, NT-proBNP), inflammation (CRP) and fibrosis (galectin-3) during anthracycline-containing chemotherapy. In addition we studied if change in these circulating biomarkers were affected by concomitant treatment with candesartan and/or metoprolol. Levels of the studied circulating cardiovascular biomarkers all increased at completion of anthracycline-containing chemotherapy (all p<0.05). Metoprolol, but not candesartan, attenuated the troponin response (cTnI: median change (IQR) 2.9 (1.6, 6.8) vs. 5.7 (2.3, 10.0) ng/L; cTnT: 3.4 (2.0, 7.4) vs. 5.0 (2.6, 9.9) ng/L; both p<0.05). There was no association between baseline and on-treatment biomarker concentrations and change in cardiac function during anthracycline therapy. Hence, circulating cardiovascular biomarker indices of myocardial injury, dysfunction, inflammation and fibrosis increased during anthracycline treatment. The troponin release was attenuated by metoprolol (Figure 12), but not candesartan.
**Figure 12:** Summarizing illustration of the main findings of paper # IV *Neurohormonal blockade and circulating cardiovascular biomarkers during anthracycline therapy in breast cancer patients: results from the PRADA study.* Cardiac troponin (cTn) I shown in blue, and cTnT in red. Values are median change with interquartile range. Panel on the left shows a dose dependent increase during anthracycline-containing chemotherapy for those with a total cumulative epirubicin dose below 400 mg/m² (range 240-360 mg/m²) and of 400 mg/m². Panel on the right shows that metoprolol attenuated the circulating level of troponins.

<table>
<thead>
<tr>
<th></th>
<th>Epirubicin &lt; 400 mg/m²</th>
<th>Epirubicin = 400 mg/m²</th>
<th>No Metoprolol</th>
<th>Metoprolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>cTnI ng/L</td>
<td>3.75 (1.58, 6.93)</td>
<td>6.40 (2.50, 13.50)</td>
<td>5.7 (2.3, 10.0)</td>
<td>2.9 (1.6, 6.8)</td>
</tr>
<tr>
<td>cTnT ng/L</td>
<td>3.59 (2.20, 7.41)</td>
<td>7.54 (3.56, 11.71)</td>
<td>5.0 (2.6, 9.9)</td>
<td>3.4 (2.0, 7.4)</td>
</tr>
</tbody>
</table>

*p<0.05

**7.5. Methodological considerations**

*Design*

A randomized controlled trial (RCT) is considered the optimal study design to evaluate the efficacy of an intervention. Randomization allows for the subjects in a study to be allocated by chance to the different groups, avoiding systematic differences. For simple randomization a table of random numbers or a random number generator on a computer is used. At any given time, with this method, the number of participants in each treatment arm will probably differ. To avoid this a block randomization can be used. This allows for a close to similar number of participants in each group at all times.
However in smaller studies randomization can still lead to imbalance for important characteristics. To avoid imbalance without sacrificing the advantage of randomization, stratified randomization can be used. Stratified randomization ensures that the groups are balanced for certain relevant characteristics. It is done by performing a separate randomization for each subgroup. Stratified randomization has to be combined with block randomization to maintain the balance of treatments. In PRADA, patients were stratified for anthracycline dose and trastuzumab. Both higher anthracycline doses and trastuzumab will have an impact on the LVEF, and only a minority will receive higher doses of anthracycline and trastuzumab.

The 2x2 factorial design allowed us to test two different drugs without increasing the sample size drastically. As others, we found no interaction between metoprolol and candesartan. Hence those assigned to metoprolol could be compared to those not assigned to metoprolol and similarly those assigned to candesartan could be compared to those not assigned to candesartan.

**Errors**

Errors can basically be divided into random errors or systematic errors. Random errors are unknown and unpredictable variations for each time a measurement is conducted. They can be evaluated through statistical analysis and can be reduced by larger sample size. Imaging analyses can be more prone to random errors due to subjective variations of areas to be included or excluded. For analyzing circulating cardiovascular biomarkers three different laboratories were used. Even though the samples were handled the same way, we cannot completely rule out the possibility of random errors related to the handling, storage and thawing.

Systematic errors consequently divert its outcomes away from true values and persist throughout the entire experiment. Hence it limits the internal validity of the study. An important part of systematic error is confounding. Confounding is a situation in which the effect or association between an exposure and outcome is distorted by the presence of another variable. Systematic errors cannot be analyzed statistically, but may be minimized by randomization (minimizing the selection bias), blinding (minimizing the performance-/detection bias) and using intention-to treat analysis (minimizing the attrition bias). The PRADA-study was randomized and blinded, and statistics were performed according to the intention-to-treat principle, hence the systematic errors were reduced to a minimum.
**External validity**

External validity refers to whether the results can be applied in a general setting. Randomized, placebo-controlled trials have strict inclusion and exclusion criteria that at times make it difficult to generalize the results to a clinical setting. This is mainly because patients outside of trials often have more comorbidities.

In paper # III and IV we specifically assessed the change in CMR indices and circulating cardiovascular biomarkers after anthracycline-containing chemotherapy. Considering the effects of the prevention of left ventricular dysfunction with Enalapril and candesartan in patients submitted to intensive Chemotherapy for the treatment of Malignant Hemopathies (OVERCOME) trial in patients with hematological malignancies\textsuperscript{78}, it could be argued that candesartan and metoprolol may have a cardioprotective effect in all patients treated with anthracycline-containing chemotherapy regardless of underlying malignancies.

### 7.6. Discussion

**Paper # I**

This is a paper of the methodology of the PRADA-study, hence no results are presented.

**Paper # II**

This paper provides the primary findings of the PRADA study. Contemporary adjuvant breast cancer treatment in women with no serious cardiovascular comorbidities was associated with a numerically modest, but statistically significant reduction in LVEF as measured by CMR. Candesartan, but not metoprolol, significantly attenuated the decline in LVEF. No beneficial effect of candesartan or metoprolol was found on the secondary endpoints; right ventricular ejection fraction, GLS, E/E’ and change in cardiac troponin levels.

Prior to PRADA there have been three other published studies on the cardioprotective effect of angiotensin antagonists with or without a beta blocker in the adult population during anthracycline-containing chemotherapy (Table 5). Two of the studies showed a beneficial effect of the neurohormonal blockade\textsuperscript{78,79} while one showed no effect\textsuperscript{80}. None of the studies included breast cancer patients exclusively and they either lacked randomization, placebo or the double-blind design.
Table 5: Treatment with angiotensin antagonists alone or in combination with a beta blocker during anthracycline-containing chemotherapy in adults

<table>
<thead>
<tr>
<th>First author (study name)</th>
<th>Intervention (n)</th>
<th>Design/Population</th>
<th>Imaging (follow-up in months)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Georgakopoulos⁸⁰</td>
<td>Metoprolol (42)</td>
<td>Design: Randomized No placebo Not blinded</td>
<td>Echo (12)</td>
<td>There was no significant difference in the change in LVEF between the control and the intervention group</td>
</tr>
<tr>
<td></td>
<td>Enalapril (43)</td>
<td>Population: Different lymphomas</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control (40)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bosch⁷⁸ (OVERCOME)</td>
<td>Enalapril and carvedilol (45)</td>
<td>Design: Randomized No placebo Not blinded</td>
<td>Echo and CMR (6)</td>
<td>Change in LVEF was significantly greater in the control group compared to the intervention group when measured by echo (n=79), but not CMR (n=58)</td>
</tr>
<tr>
<td></td>
<td>Control (45)</td>
<td>Population: Malignant hemopathies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dessi⁷⁹</td>
<td>Telmisartan (25)</td>
<td>Design: Randomized Placebo controlled Single blind</td>
<td>Echo (12)</td>
<td>Lower strainrate in the placebo group compared to intervention group</td>
</tr>
<tr>
<td></td>
<td>Placebo (24)</td>
<td>Population: Heterogeneous cancer population</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Echo echocardiography; OVERCOME prevention of left Ventricular dysfunction with Enalapril and carvedilol in patients submitted to intensive ChemOtherapy for the treatment of Malignant hemopathies

The reason for the discrepancy between the studies may be the design, use of different imaging methods or use of different cardioprotective treatment regimens.

Interestingly, during the same time period as the PRADA trial there were two studies examining the cardioprotective effect of angiotensin antagonists during trastuzumab treatment⁸¹,⁸². The Multidisciplinary Approach to Novel Therapies in Cardio-Oncology Research (MANTICORE) study was randomized, placebo-controlled and double-blind, and used CMR as the imaging modality. There was no
difference in change in diastolic volume, which was the primary endpoint. However there was a significant decline in LVEF which was attenuated by the angiotensin converting enzyme (ACE) inhibitor perindopril. The study by Boekhout et al was also randomized, placebo-controlled and double-blind, but used MUGA and not CMR as the imaging modality. In this study candesartan did not attenuate the decline in LVEF during trastuzumab treatment.

Animal studies on pathophysiology of anthracycline-induced cardiotoxicity have shown involvement of angiotensin II receptors. Angiotensin II type 1a receptor knockout mice or those treated with angiotensin II receptor blockers were protected from anthracycline-induced cardiotoxicity. For trastuzumab-induced cardiotoxicity, angiotensin II plays an additional role to the HER2 receptors. Angiotensin production is upregulated during cardiac stress and down-regulates neuregulin (NRG)-1 leading to less activation of HER receptors and cell survival pathways as described in Figure 3. Angiotensin II binding to angiotensin II type 1 receptors on the cardiomyocytes also leads to nicotinamide adenine dinucleotide phosphate oxidase (NADPH) activation which is harmful for the heart. Hence ARBs protect trastuzumab-induced cardiotoxicity via multiple mechanisms.

The lacking effect of candesartan on the secondary end-points in the PRADA study may have been an issue of power and timing of blood samples. For instance cancer therapy induced cardiotoxicity is often divided into type 1 and type 2 cardiotoxicity. Anthracyclines commonly causes type 1 cardiotoxicity with myocyte death while trastuzumab typically causes type 2 cardiotoxicity with myocyte dysfunction. In the PRADA study end-of-study for the majority of patients were either after radiation or trastuzumab. If the level of circulating cardiovascular biomarkers peaked after anthracycline-containing chemotherapy, they would have been low after trastuzumab and radiation therapy.

Prior to PRADA there have been four other published studies on the cardioprotective effect of beta blockers with or without an angiotensin antagonist in the adult population during anthracycline-containing chemotherapy (Table 6). Three of the studies showed beneficial effect of a beta blocker with or without an angiotensin antagonist. None of the studies used CMR. Only one included breast cancer patients exclusively and was randomized, placebo-controlled and double-blind. The reason for the discrepancy of the results may be the varied populations and the use of different beta blockers.
Table 6: Treatment with a beta blocker alone or in combination with an angiotensin antagonist during anthracycline-containing chemotherapy in adults

<table>
<thead>
<tr>
<th>First author (study name)</th>
<th>Intervention (n)</th>
<th>Design/Population</th>
<th>Imaging (follow-up in months)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kalay85</td>
<td>Carvedilol (25)</td>
<td>Design: Randomized Placebo controlled Single blinded</td>
<td>Echo (6)</td>
<td>LVEF decline was attenuated in carvedilol group.</td>
</tr>
<tr>
<td></td>
<td>Placebo (25)</td>
<td>Population:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heterogeneous</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>cancer population</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kaya86</td>
<td>Nebivolol (27)</td>
<td>Design: Randomized Placebo-controlled Double-blind</td>
<td>Echo (6)</td>
<td>LVEF decline was attenuated in the nebivolol group</td>
</tr>
<tr>
<td></td>
<td>Placebo (18)</td>
<td>Population:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Breast cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Georgakopoulos80</td>
<td>Metoprolol (42)</td>
<td>Design: Randomized No placebo Not blinded</td>
<td>Echo (12)</td>
<td>There was no significant difference in change in LVEF between the control and the intervention group</td>
</tr>
<tr>
<td></td>
<td>Enalapril (43)</td>
<td>Population:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control (40)</td>
<td>Different lymphomas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bosch78 (OVERCOME)</td>
<td>Enalapril and</td>
<td>Design: Randomized No placebo Not blinded</td>
<td>Echo (6)</td>
<td>Change in LVEF was significantly greater in the control group compared to intervention group when measured by echo (n79), but not CMR (n=58)</td>
</tr>
<tr>
<td></td>
<td>carvedilol (45)</td>
<td>Population:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control group (45)</td>
<td>Malignant</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>hemopathies</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Echo echocardiography; OVERCOME preventiOn of left Ventricular dysfunction with Enalapril and caRvedilol in patients submitted to intensive ChemOtherapy for the treatment of Malignant hEmopathies

MANTICORE is hitherto the only study published after the PRADA study evaluating the effect of a beta blocker during breast cancer treatment. The beta blocker bisoprolol attenuated the decline in LVEF compared to placebo as measured by CMR in those treated with trastuzumab. However, as mentioned previously, change in diastolic volume and not LVEF was the primary endpoint81.
There are animal studies arguing for both the beneficial effect of selective and non-selective beta blockers with or without antioxidative properties. As the anti-cardiotoxic effect of beta blockers may be mediated via the inhibition of beta-adrenergic mediated proapoptotic pathways during anthracycline treatment, the effect of beta blockers may become evident during longer-term follow-up.

Longer-term follow-up will be of importance before any certain clinical implications can be made from the PRADA-study. From previous interventional studies preventing negative cardiac remodeling, we know that a numerical modest change in LVEF may be associated with larger clinical events on follow-up.

Paper # III
This paper reports on subclinical structural myocardial changes associated with anthracycline treatment, detectable by novel CMR parameters. Higher doses of epirubicin were associated with a greater increase in ECV fraction and total ECV, both indices of edema and diffuse myocardial fibrosis. Candesartan both alleviated the decline in LVEF and was associated with a greater decline in total cellular volume. No such effect was seen of metoprolol.

Anthracycline-induced cardiotoxicity is associated with diffuse myocardial fibrosis. Commonly, cardiotoxicity is measured as LV systolic function and not by morphological changes. Novel CMR parameters now allow detecting myocardial pathophysiological processes non-invasively. ECV fraction can increase as a result of expansion of the extracellular space either by edema or fibrosis, or by reduced myocyte volume (Figure 13).
ECV fraction is a surrogate for myocardial fibrosis and increasing fibrosis is associated with cardiac dysfunction. Only sparse data exist on ECV fraction and anthracycline-induced cardiotoxicity. In a study of pediatric patients followed with CMR for two years or more after anthracycline exposure, ECV fraction increased compared to healthy controls. This was also related to cumulative anthracycline dose, exercise capacity and myocardial wall thinning. In an adult heterogeneous cancer population, Melendez et al showed that ECV increased from baseline to three months in those treated with an anthracycline based chemotherapy regimen compared to those treated with a none anthracycline based chemotherapy regimen.

Whether the increase in ECV is due to reduced myocyte volume or expansion of extracellular space, can be determined by relating ECV fraction to mass. For instance in a hypertension study of renal denervation there was no increase in ECV fraction six months later. However, there was a significant reduction in LV mass and total ECV suggesting the mass reduction was due to reversal of both myocyte hypertrophy and interstitial myocyte fibrosis. There are no studies reporting the longitudinal change of total ECV during anthracycline-based chemotherapy in a homogenous cancer population. In paper # III we showed a dose dependent increase of both ECV fraction and total ECV. As there was no dose dependent difference of the total cellular volume, the increase in both ECV fraction and total ECV was probably related to an increase in the extracellular volume. Whether this increase was because of increased interstitial fibrosis or edema cannot be determined by the data available. However, a recent animal study demonstrated edema by CMR and histopathology five weeks after initiation of high-dose anthracycline treatment followed by fibrosis at 10 weeks.

---

Figure 13: A. Showing normal extracellular volume (ECV) fraction in the myocardium. B. ECV fraction is increased due to increased total ECV. C. ECV fraction is increased due to decreased total cellular volume.
Further we showed that total cellular volume was reduced in those treated with candesartan compared to those not treated with candesartan. Candesartan did not affect the ECV fraction or total ECV. An explanation may be the impact of candesartan on the intracellular fluid balance and protein synthesis leading to reduced total cellular volume and mass\textsuperscript{96-98}. In summary, inhibition of the renin-angiotensin-aldosterone system in the setting of anthracycline therapy certainly has complex actions on cardiomyocyte structure and function.

Paper # IV

In this paper circulating cardiovascular biomarkers reflecting different pathophysiological mechanisms were measured serially during anthracycline therapy. Treatment with contemporary anthracycline doses for early breast cancer was associated with increase in cTnI and cTnT (indices of myocardial injury), BNP and NT-proBNP (indices of cardiac dysfunction), CRP (index of inflammation) and galectin-3 (index of fibrosis). The increase was not associated with early decline in ventricular function. Metoprolol, but not candesartan, attenuated early myocardial injury.

There are conflicting results in the literature whether the level of these circulating cardiovascular biomarkers increase during anthracycline treatment\textsuperscript{44}. This may be explained by the heterogeneity of populations studied, type and dosage of anthracyclines used, timing of blood samples, and importantly sensitivity of biomarker assays used. There is sparse data on how beta blockers and angiotensin antagonists affect the levels of circulating cardiovascular biomarkers. In paper # II and III we showed that candesartan, but not metoprolol had a preventive effect on LV systolic function during breast cancer treatment as measured by CMR. In paper #IV metoprolol, but not candesartan, attenuated the change in troponin response. This may seem contradictory to the results concerning the effect of candesartan and metoprolol on LVEF. However studies have shown that the potential mechanism mediating this anti-cardiotoxic effect is the inhibition of beta-adrenergic mediated proapoptotic pathways. Animal studies have shown that there is a non-specific interaction of anthracyclines with cardiac beta-adrenergic receptors and that beta adrenergic receptor 1 is linked to proapoptotic pathways\textsuperscript{99,100}. Bernstein et al showed that beta 1 adrenergic receptor knockout mice were protected against cardiotoxicity\textsuperscript{87}. In other words, a selective beta 1 antagonist as metoprolol may have an immediate effect on the cardiac apoptotic effect of anthracyclines. On the other hand, candesartan affects ventricular remodeling by
counteracting the stimulation of the collagen synthesis stimulated by angiotensin II\textsuperscript{101}. As candesartan does not affect the proapoptotic pathways this may explain why it does not affect the change in levels of troponin.

Of the studies using neuro-endocrine blockade during anthracycline-containing chemotherapy to prevent cardiotoxicity only two included one or more circulating cardiovascular biomarker also measured in the PRADA study\textsuperscript{78,86}. In the OVERCOME study the participants were randomized to either the combination with the beta blocker carvedilol and the ACE-inhibitor enalapril or to a control group\textsuperscript{78}. No significant difference was observed in the levels of circulating cTnI or BNP in the two groups. However in the OVERCOME study high sensitivity assays were not used. For cTnI Advia Cenature CP was used and the cut off level was 40 ng/L corresponding to the 99 percentile. BNP was measured using Advia Centaur Immunochemistry analyzer by Siemens.

Kaya et al randomized the patients to either the beta blocker nebivolol or placebo and found an increase in NT-proBNP in the control group but not in the beta blocker group\textsuperscript{86}. Conversely, in the PRADA-study NT-proBNP increased in the beta blocker group. The increase of natriuretic peptides in the beta blocker group in the PRADA study was not surprising as beta blockers have shown to increase natriuretic peptide concentrations in healthy subjects as well as in a variety of clinical settings\textsuperscript{102-104}.

The discrepancy between the two studies may be that Kaya et al had a more comorbid population that may have benefited from the beta blocker regardless of chemotherapy treatment. Another explanation may be that the cumulative anthracycline dose was higher in the study by Kaya et al.

We found no association between baseline and on-treatment biomarker concentrations and change in LVEF during anthracycline therapy. Longer-term follow-up will be required to determine whether there are any long-term associations between changes in biomarkers and change in LVEF. An important question to consider is the timing of biomarker sampling. To the best of our knowledge the kinetics of the biomarkers used in this study have not been investigated during anthracycline treatment in humans. However others have shown a persistent elevation of troponin months after completed anthracycline-containing chemotherapy\textsuperscript{51}. It could therefore be argued that the release of biomarkers in these patients is an ongoing process. In the PRADA study levels of all measured biomarkers were significantly higher post- than pre-
anthracycline treatment, indicating that the sampling timing was within the elevated level of these biomarkers. We cannot rule out that another timing of blood sampling would have resulted in higher biomarker concentrations. Probably, each circulating biomarker would have peaked at a unique time. However, daily blood sampling would not have been logistically feasible or ethically acceptable.

7.7. Strengths and limitations
The main strength of the PRADA study was the randomized, placebo-controlled, double-blind design. The 2x2 factorial design permitted a head to head comparison of two drugs without increasing the participant numbers drastically. Another strength, compared to previous studies, was the homogenous breast cancer population and the use of CMR, the gold standard for measuring LVEF. Finally the PRADA population had low rates of comorbid conditions. Hence the cardiotoxicity measured could directly be related to the cancer treatment. Certainly this could also be seen as a limitation. However, if the prevalence of cardiovascular comorbidities had been high, there probably would have been indication for the use of beta blockers or angiotensin antagonists regardless of chemotherapy treatment. Another limitation was the single center design. The study sample was relatively small, but still PRADA was the largest study in cardio-oncology of its time. The complex end-of-study definition (Figure 9) could also be seen as a limitation, but the main purpose of this study was to evaluate the cardiotoxic effect of contemporary adjuvant breast cancer treatment regardless of treatment regimen.

Substudies from the primary study were used to evaluate the effect of anthracyclines. The sample size of these studies was calculated based on the anticipated effect of intervention on the primary outcome measure. It is therefore likely that power calculation using secondary or tertiary variables of the study may have resulted in other sample sizes. However, all variables to be measured and their hierarchy were predefined in a statistical analysis plan.

7.8. Conclusions
The main purpose of this thesis was to study whether candesartan and/or metoprolol could prevent cardiotoxicity induced by early breast cancer treatment. Additionally, we wanted to assess the effect of neuroendocrine blockade during anthracycline therapy as
measured by novel CMR parameters and circulating cardiovascular biomarkers. In the first paper we described the study. In the second paper we showed that candesartan, but not metoprolol attenuated the decline in LVEF at end of breast cancer treatment. However, the clinical implication of this can only be determined when longer-term data are available. In the third and fourth paper we evaluated the cardiotoxic effect of anthracycline-containing chemotherapy. The novel CMR parameters for edema and myocardial fibrosis as ECV fraction and total ECV, showed an anthracycline dose dependent increase. Candesartan, but not metoprolol attenuated the decline in LVEF and was associated with a greater decline in total cellular volume. Finally, circulating cardiovascular biomarkers such as cardiac troponins, BNP, NT-proBNP, galectin-3 and CRP, indices of cardiac injury, dysfunction, fibrosis and inflammation, increased during anthracycline treatment. In this cohort metoprolol, but not candesartan attenuated the increase in troponin concentrations.

7.9. Clinical implications and future research
No certain clinical implications can be made from the PRADA study. PRADA showed a numerically modest, but statistical significant decline in LVEF during breast cancer treatment. Candesartan and to some degree metoprolol may have a cardioprotective role. Longer-term follow-up is necessary to determine this initial positive effect of candesartan and metoprolol.

Future RCTs should focus on a population with higher risk of cardiac disease. Beta blockers and angiotensin-antagonists have shown some promising cardioprotective effect and should be explored thoroughly. Other drugs as statins and aldosterone antagonists are also being tested\textsuperscript{105-107}, and should also be explored thoroughly. As cardio-oncology clinics are becoming more common, publication of observational data will be of importance to determine the real life experience of cardiotoxic effect of contemporary breast cancer treatment.

7.10. Closing remarks
In Norway you are not supposed to brag. In India it’s compulsory to brag. As I do have Indian genes I will spare a short paragraph to bragging. I will not lie, working the logistics of PRADA was not easy, but people all along the way have been more than helpful. My constant presence at the breast cancer surgical and oncological out-patient clinic made the staff there sure not to forget recruiting and follow-up on the PRADA
patients. By the end of the recruitment period, the staff at the surgical out-patient clinic could often exclaim “oh, you are here! We have patients who are eligible for chemotherapy!” The secretaries, feeling sorry I had to spend so much time waiting in the corridor, started bringing me coffee. On days I just walked past for other reasons, they would kindly tell me that no breast cancer patients were scheduled for the day. The staff at the oncology out-patient clinic must have known my phone number by heart, because by the end of the trial time they called me before the oncologists to discuss the patient’s blood pressure.

The acronym PRADA has been a good ice-breaker, and put a smile on many faces. Certainly, that the project was focusing on women with a potentially curable disease with a “heart breaking” side effect also helped.

PRADA has received considerable attention since we started. In 2012 I participated at the Norwegian Research Grand Prix and won the competition in Oslo. More importantly the primary results of PRADA were presented at the late-breaking clinical trial session at American Heart Association (AHA) Scientific Session in November 2015. A summary from the media report in January 2016 indicated our late-breaking abstract topped 2.1 million hits. The study was mentioned in at least 14 different articles/interviews. At the European Society of Cardiology congress in February 2016, Dr. McMurray summarized the publications in European Heart Journal for 2015. PRADA was mentioned among the journals top 10 articles. Finally PRADA was also reported under “big discovery” on the flyer for the AHA Scientific Session 2016 (Figure 14). Still, most importantly I have now been part of establishing the first cardio-oncology outpatient clinic in Norway at Akershus University Hospital. With these final remarks, I end my thesis!
**Figure 14:** Flier for American Heart Association Scientific Sessions 2016. PRADA is mentioned under “big discovery”, encircled in red
8. References

5. www.nbcg.no. (23.06.17)
13. http://nbcg.no/content_l/text_37a64756-6362-4839-87a0-245f4946a935/1486391674129/oversikt_nbcg_retnningslinjer_adjuvant_systemisk_behandling_01_02_17.pdf (23.06.17)
19. http://nbcg.no/behandlingsskjemaer/content/text_cd48dbb7-a02b-4c8f-8898-1916778da89/1405967931633/algoritme_for_dis_kontinuering_av_trastuzumab_basert_p_lvef.pdf (23.06.17)
74. Cohn JN, Tognoni G, Glazer RD, Spormann D, Hester A. Rationale and design of the Valsartan Heart Failure Trial: a large multinational trial to assess the
76. https://onlinecourses.science.psu.edu/stat507/node/34 (04.08.2017)
