Cardiotoxicity after anticancer treatment

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General introduction and aims of the thesis

Breast cancer is the most common cancer in females in the western world, and the second most common cause of cancer death in women (after lung cancer). In the Netherlands, it has an incidence of approximately 130/100,000 women per year (126.7 per 100,000 women in 2001),[1] and is the main cause of death in women aged 45 to 55 years (1294 women aged 40 to 64 years died as a result of breast cancer in The Netherlands in 2001).[2] Due to improvement in local and systemic treatment, the survival of patients with breast cancer is increasing. Consequently, the incidence of long-term side effects of antineoplastic treatment is becoming to be more relevant. Cardiac toxicity is a well known adverse effect observed with several cytostatic agents (reviewed in [3]) and chest wall irradiation. Chest wall irradiation has been suggested to be an additional risk factor for the development of anthracycline-induced cardiotoxicity.[4,5]

Anthracyclines

Anthracyclines are particularly are well known for their cardiotoxic side effects. These cardiovascular complications can occur acute (during administration), early (several days to months following administration),[5] or late (years to decades following exposure). Acute cardiotoxicity is uncommon, but can consist of arrhythmias, left ventricular dysfunction, a peri-myocarditis syndrome or electrocardiographic abnormalities.[6] Early cardiotoxicity generally expresses itself as heart failure and is related to the cumulative anthracycline dose. The incidence of early cardiotoxicity particularly increases when the cumulative dose exceeds a certain threshold. For doxorubicin this is 550 mg/m^2, for epirubicin 900-1000 mg/m^2, for daunorubicin 550 mg/m^2, and for mitoxantrone 160 mg/m^2.[7] Late cardiotoxicity can occur up to years after treatment and consists of heart failure due to non-ischemic dilated cardiomyopathy,[8] which is considered to be irreversible.[9] This chronic cardiotoxicity is clinically the most relevant type.[10] Data concerning the prevalence of late cardiotoxicity is limited and mostly derived from studies performed in childhood cancer survivors. In a cross-sectional study in 229 anthracycline-treated childhood cancer survivors, heart failure was diagnosed in 10%, and asymptomatic left ventricular dysfunction (echocardiography) in an additional 10%, 15 years after treatment.[11] The most important risk factors that have been identified for the development of anthracycline-induced cardiotoxicity are high cumulative doses in particular, age over 70 years, radiotherapy to the cardiac region, underlying cardiac disease, concomitant administration of other antineoplastic agents.[5] In addition, more severe subclinical cardiotoxicity has been reported for male patients or patients who are overweight.[12]

During the last decade, the use of anthracyclines has especially increased in the adjuvant setting in breast cancer. As a result, the issue of late cardiac toxicity can limit breast cancer patients' quality of life and even survival, and is therefore becoming an increasingly important issue the total treatment of these cancer patients. The therapeutic effect of anthracyclines is mediated by their insertion into DNA in replicating cells, causing DNA fragmentation, inhibition of polymerases,

and decreased DNA, RNA and protein synthesis. This mechanism is unlikely to be involved in myocardial injury, since myocyte replication is, at least, less active. Myocyte damage has been attributed to the production of toxic free oxygen radicals and an increase in oxidative stress, which cause lipid peroxidation of membranes, leading to vacuolation, irreversible damage and myocyte replacement by fibrous tissue.[13] In rat cardiomyocytes, doxorubicin and exposure to free radicals induces cyclooxygenase-2 (COX-2) activity, which in turn, limits doxorubicin-induced cardiac cell injury.[14] Inhibition of COX-2 activity potentiates doxorubicin-mediated cardiac injury in vivo.[15] Doxorubicin is also associated with a decrease of the endogenous antioxidant enzymes, such as glutathione peroxidase, that are responsible for the scavenging of free radicals.[16]
Animal models are particularly interesting to study the mechanisms and pharmacological intervention of cardiotoxicity induced by anthracyclines. Simunek et al. for instance characterized a rabbit model of daunorubicin-induced cardiotoxicity, in which they evaluated the cardioprotective effects of the free-radical scavenger dexrazoxane.[17] Cardiac function was assessed by measuring serum troponin T levels, histological examination of cardiac tissue, invasive hemodynamic measurement of left ventricular systolic and diastolic pressure parameters, and echocardiography to assess the left ventricular ejection fraction (LVEF). They observed that dexrazoxane treatment limited the cardiotoxic effects induced by daunorubicin.[17]

Other antineoplastic agents
Next to the anthracyclines, several other antineoplastic agents can induce cardiac toxicity. However, the incidence is low, mostly unpredictable and the occurrence is not related to the cumulative dose in most cases. Cyclophosphamide for instance, can lead to cardiac dysfunction. However, this is almost exclusively encountered with administration of high doses, such as used before bone marrow or stem cell transplantation.[18] Fluorouracil is another agent that is associated with cardiotoxicity, with symptoms varying from cardiac arrhythmias, silent myocardial ischemia, angina, congestive heart failure and even sudden death.[19,20] Bradycardia and heart block have been observed with paclitaxel, but the clinical significance of these cardiotoxic events is unclear. Transient asymptomatic bradycardia has been reported in 29% of patients, while profound cardiac disturbances, including atrioventricular conduction block, ventricular tachycardia, and manifestations of cardiac ischemia, are observed in 5% or less.[21] Most of these side effects were asymptomatic. However, paclitaxel used in combination with doxorubicin was found to increase the risk of anthracycline-induced heart failure, as was observed in 18% of the women treated with this combination for metastatic breast cancer.[22]
After adjuvant chemo- and radiotherapy, breast cancer patients receive additional endocrine maintenance therapy on indication, which until recently mostly consisted of tamoxifen. Tamoxifen has not been associated with cardiotoxicity. Nevertheless, an increased risk of thromboembolic complications is observed with this agent.[23]

Aromatase inhibitors, which are currently often used instead of tamoxifen, are less frequently associated with cardiovascular complications.

Trastuzumab

Although the use of classical chemotherapeutic agents has improved the prognosis of breast cancer patients, still many patients die from this disease and the need for newer and more effective agents remains. The field of molecular biology has advanced dramatically in recent years and has yielded many new insights regarding molecular characterization of breast cancer. The human epidermal growth factor receptor 2 (HER2, also known as erbB-2) for instance, is a transmembrane tyrosine kinase receptor that induces cellular growth and survival when activated. HER2 overexpression or gene amplification, which is present in the tumors of 25-30% of breast cancer patients, adversely affects the prognosis of these patients.[24] Trastuzumab is a monoclonal antibody against HER2. The use of trastuzumab either as a single agent,[25] or in combination with chemotherapy can improve survival of patients with metastatic disease of HER2-positive breast cancer.[26] A retrospective analysis of a phase III study however, revealed an increased incidence of heart failure, especially among patients treated with the combination of doxorubicin-cyclophosphamide and trastuzumab (27%); in the patients treated with paclitaxel and trastuzumab, heart failure occurred in 13%.[26] Single agent trastuzumab resulted in only 4% cardiotoxicity.[27] At present, no long-term follow-up data regarding trastuzumab-related cardiotoxicity are available.

The first evidence indicative of the presence of HER2 in the heart, stems from mice deficient in HER or neuregulin, a group of HER-activating ligands. These mice die during embryonic development due to severe neurologic and cardiac abnormalities.[28-32] Cardiomyocyte-expressed HER2 is critically important in the cardiac development in rodents,[33] and lack of the receptor in cardiomyocytes irrevocably leads to the development of severe dilated cardiomyopathy.[34] It can be postulated that trastuzumab exerts a direct effect on cardiomyocyte membrane-expressed HER2. *In vitro* addition of trastuzumab to cardiomyocytes that lacked HER2, resulted in increased apoptosis in these cells.[34] It can be imagined that increased cardiomyocyte apoptosis, through lack of growth promoting signaling, underlies trastuzumab-related cardiotoxicity.

DETECTION OF CARDIOTOXICITY

Imaging techniques

Currently, LVEF measurement, performed by multigated angiography (MUGA) or ultrasound, is generally accepted as the method of choice to detect cardiotoxicity of anticancer treatment. The LVEF reflects the functional status of the left ventricle. This implies that subclinical myocardial injury, not affecting pump function of the heart, will not be detected by LVEF measurement. Clinicians are trying to find

methods to identify patients who will develop cardiotoxicity of anticancer treatment, in order to be able to intervene earlier and prevent or limit clinical symptoms.
One potential new diagnostic method for the early detection or prediction of trastuzumab-related cardiotoxicity may be pre-treatment scintigraphy with radiolabeled trastuzumab as the radioactive tracer. It can be imagined that the cardiotoxic effects of trastuzumab are a consequence of direct binding of trastuzumab to HER2 expressed in the myocardium. In a preliminary report, Behr and colleagues suggested that trastuzumab could be radiolabeled effectively and that pre-treatment radiolabeled trastuzumab scintigraphy could be used to predict trastuzumab-related cardiac toxicity and tumor response.[35]

Natriuretic peptides
Biochemical markers that are established indicators of cardiac functional status in patients with heart failure, may also be indicative of anticancer treatment-related cardiotoxicity. The natriuretic peptides are neurohormones which are increased in case of cardiac dysfunction; N-terminal atrial natriuretic peptide (NT-ANP), a linear peptide from ANP, is mainly derived from the cardiac atria and B-type natriuretic peptide (BNP) is secreted by the ventricles. In the emergency setting of acute dyspnea, increased plasma BNP levels could discriminate between left ventricular dysfunction and non-cardiac causes for the dyspnea.[36] Furthermore, increased natriuretic peptide levels have been related to decreased LVEF values in patients with left ventricular dysfunction or coronary artery disease.[37,38] Meinardi et al. observed a progressive increase in plasma levels of N-terminal atrial natriuretic peptide (NT-ANP) and B-type natriuretic peptide during the first year after chemotherapy in 40 breast cancer patients treated with moderate-dose (either 360 or 450 mg/m^2) epirubicin-containing chemotherapy and chest wall irradiation. This increase was first present one month after the start of chemotherapy. Although mean natriuretic peptide levels remained within normal ranges, and no association with symptomatic heart failure or a decrease in LVEF was observed, the increase in natriuretic peptide plasma levels was considered to be suggestive of myocardial injury.[39] In a study among breast cancer patients who received either 9 cycles of fluorouracil, epirubicin and cyclophosphamide (median epirubicin dose 774 mg/m^2, n=49) or 3 cycles of fluorouracil, epirubicin and cyclophosphamide (median epirubicin dose 181 mg/m^2, n=56) followed by high-dose cyclophosphamide, thiotepa and carboplatin, plasma proANP levels were measured up to 3 years after treatment and were higher in the 9xFEC group and correlated with the total epirubicin dose.[40]

Troponins
The cardiac troponins I and T, proteins that are exclusively present in myocardial cells, are other examples of plasma markers of cardiac injury. The troponin I plasma concentration is a well-established specific and sensitive marker of

myocardial injury, with both high diagnostic and prognostic value.[41] In a recent report, the troponin I release pattern was measured soon after chemotherapy (early) and one month later (late), in patients with different types of malignancies who received treatment with high-dose chemotherapy, consisting of regimens with or without an anthracycline derivative. Patients who had a positive troponin I value both early and late, had a high risk (84%) of a cardiovascular event (i.e. cardiovascular death, pulmonary edema, (a)symptomatic left ventricular dysfunction, rhythm disturbances), compared to patients with a positive early, but negative late troponin I (37%) and patient in the negative troponin I group (1%).[42]

Circulating apoptotic proteins

In patients with heart failure unrelated to anticancer treatment, cardiomyocyte apoptosis is increased.[43,44] Plasma soluble apoptosis-related proteins belonging to the tumor necrosis factor (TNF)-superfamily of proteins, among which are TNFα, TNF-Receptor 1 and 2, Fas and Fas ligand, are elevated in heart failure and correlate positively with New York Heart Association (NYHA) functional class.[45-48] It can therefore be proposed that these cytokines are also of use in the detection of cardiac injury in patients who have been treated with anti-neoplastic agents.

The extracellular domain of the transmembrane HER2 receptor can be proteolytically cleaved from the cell membrane and shed into the circulation. HER2 can be quantified in serum samples with an enzyme-linked immunosorbent assay (ELISA). In patients with HER2-positive breast cancer, serum HER2 levels are increased and correlate positively with metastatic tumor burden.[49] Bearing in mind the crucial role of HER2 in the animal heart, we hypothesized that trastuzumab treatment may result in attenuated growth promoting signaling, normally provided for by HER2 signal transduction. From a more mechanistic point of view, we hypothesized that heart failure develops when HER2 transmembrane signaling falls short, which may be related to a change in serum HER2 concentrations.

AIMS OF THE THESIS

The aim of this thesis is to evaluate the potential role of new serological methods, such as measurement of plasma apoptotic protein and natriuretic peptide levels, in addition to imaging techniques, e.g. radiolabeled trastuzumab scintigraphy, for the detection of (sub)clinical cardiac dysfunction in breast cancer patients treated with anthracyclines.

In order to provide more insight into the role of HER2 in the pathophysiology of heart failure, serum HER2 levels in patients with heart failure will be evaluated.

Apoptotic pathways are extensively studied to develop new strategies for inducing cell death in cancer cells. New drugs are being developed that specifically target key components involved in tumor cell apoptosis. However, a major concern is that

these new compounds increase apoptosis in other organs, such as the heart. Although the role of apoptosis in the pathophysiology of heart failure is still controversial, the discovery of previously unknown apoptotic pathways in cancer research may place cardiac apoptosis in another perspective.

In **chapter 2** an overview of the literature with regard to the molecular mechanisms that are considered to be responsible for apoptosis in the heart will be presented. Next to the more accepted apoptotic pathways, the role of (lack of) the HER family of growth factor receptors in the heart is discussed.

Chapter 3 represents an editorial comment on an animal model of anthracycline-induced heart failure,[17] which might become of use for testing the cardiotoxic effects of anthracyclines and evaluating pharmacological intervention in the process of cardiac injury by anthracyclines.

We have developed a method to bind trastuzumab to the gamma-emitting isotope ^{111}Indium. In **chapter 4**, we describe this radiolabeling process and the validation of the radioactive tracer in an animal xenograft model before applying this technique in patients.

Subsequently, we investigated whether radiolabeled trastuzumab scintigraphy in patients with HER2-positive metastatic breast cancer could be used to identify patients prone to cardiotoxicity when treated with trastuzumab and paclitaxel. In this study, we also evaluated if tumor uptake of the trastuzumab tracer was present in metastatic lesions in these patients and if so, if tumor uptake varied in and between patients. The results of this study are presented in **Chapter 5**.

In **chapter 6**, the results of the measurement of serum HER2 levels in patients with heart failure due to either ischemic heart disease or dilated cardiomyopathy, not related to anticancer treatment, are described.

In addition to the studies aimed at the prediction or early detection, and understanding of trastuzumab-related cardiotoxicity, we evaluated the use of circulating neurohormones and TNF-related apoptotic proteins for early detection of anthracycline-induced (sub)clinical cardiac injury. Following up on the findings presented by Meinardi et al., who observed an increase in natriuretic peptide plasma levels during the first year following anthracycline-containing chemotherapy,[39] we studied plasma NT-ANP and BNP after extended follow-up in similarly treated patients. The results are presented in **chapter 7**.

In **chapter 8**, the evaluation of the potential applicability of plasma soluble apoptosis-related proteins for detecting cardiotoxicity after adjuvant moderate-dose epirubicin and chest wall irradiation in breast cancer patients is described. Since patients received either standard-dose or high-dose myeloablative chemotherapy followed by hematopoietic stem cell rescue, we also evaluated if the treatment was of influence on the plasma apoptosis marker levels.

Subsequently, we prospectively studied the pattern of the soluble apoptosis marker levels during the first year following the start of the same antineoplastic treatment in patients with high-risk breast cancer, as is reported in **Chapter 9**.

In **chapter 10** some perspectives for future research will be presented and a summary of the results of all investigations is given in English in **chapter 11** and in Dutch in **chapter 12**.

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