Cardiotoxicity after anticancer treatment

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Summary

The efficacy of therapy for cancer has improved dramatically in recent years. Due to the increased prognosis, it becomes critically important to limit treatment-related comorbidity in cancer survivors. Several therapeutic strategies can be applied for the treatment of patients with cancer, of which surgery, radiotherapy and chemotherapy are the most widely used. Each of these treatment modalities can lead to acute and late side effects. Chest wall irradiation and chemotherapy have been linked with an increased incidence of cardiac adverse effects. Understanding and early detection of these effects is crucial to their effective prevention and management.
Next to chest wall irradiation, several systemic antineoplastic agents are associated with the risk of developing heart failure, among which the anthracycline derivatives are the most well known. New molecular targeted agents, such as trastuzumab (Herceptin®), can also induce heart failure, and although the incidence of left ventricular dysfunction is limited when administered alone, the combination of trastuzumab with an anthracycline derivative is particularly notorious.
Next to chest wall radiotherapy, many breast cancer patients are treated with anthracycline-based chemotherapy. In addition, the use of trastuzumab in the treatment of patients with breast cancer is increasing. As a consequence, patients treated for breast cancer have an increased risk of developing antineoplastic treatment-related cardiac dysfunction.

The aim of the thesis as formulated in **chapter 1**, was to evaluate new methods and biochemical markers in the prediction or early detection of cardiotoxicity of antineoplastic treatment, and to provide more insight into the role of HER2 in the pathophysiology of heart failure.

Chapter 2 is an editorial comment on a report concerning a rabbit model for anthracycline-induced cardiotoxicity, which can be used for pharmacological testing. In the original article, the authors describe that heart failure induced by daunorubicin could be quantified adequately with cardiac catheterization, phonocardiography, echocardiography, biochemistry, and histological examinations. Although the rabbits were more susceptible to daunorubicin-induced cardiotoxicity than humans, the severity of cardiotoxicity was dependent on the cumulative dose of daunorubicin, as is the case with anthracycline-treatment in patients. Anthracyclines are among the most effective anticancer compounds and are therefore widely used in current antitumor therapy. In both curative and palliative settings, use of anthracyclines is increasing, for example in the adjuvant breast cancer treatment. The described animal model can be of use for testing pharmacological interventions aimed at prevention or amelioration of cardiotoxicity and/or preservation of cardiac function, for instance by free radical scavengers.

In **chapter 3**, the literature with regard to mechanisms of apoptosis in the heart is reviewed. As is generally accepted, apoptosis can occur through activation of the intrinsic and extrinsic pathway, or through lack of growth-promoting signaling provided for by the human epidermal growth factor family of receptors. Intrinsic or mitochondrial apoptosis occurs when a stimulus induces cytochrome c release from mitochondria. Extrinsic apoptosis is mediated by specific ligand binding to membrane receptors, initializing intracellular signaling resulting in cell death. Next to the intrinsic and extrinsic pathways, apoptosis can also be initiated through lack of growth factor receptor-mediated signaling, leading to loss of the survival promoting signal. The human epidermal growth factor receptor family consists of 4 members (HER1-4), of which all subtypes, except HER3, are expressed in the adult heart. Next to their expression in the heart, members the HER family of receptors can also be expressed in tumor cells. In anticancer treatment, new targeted therapies with monoclonal antibodies against one or more members of this family of receptors, such as trastuzumab against HER2 in breast cancer patients, are effective and are therefore used increasingly. The downside of the use of these new drugs however, is that they may also inhibit the survival promoting signaling of these receptors in the heart. The use of trastuzumab is associated with an increased incidence of heart failure. Additionally, cyclooxygenase 2 (COX2, the rate-limiting enzyme in prostaglandin synthesis) is involved in HER-related survival promoting signal transduction. Inhibition of COX-2 may therefore result in apoptosis, which may be a partial explanation for cardiovascular problems, observed with the use of selective COX-2 inhibitors, which recently caused a great deal of commotion. Better understanding of the apoptotic pathways in the heart can contribute to the safer use of anticancer agents and may lead to improved therapies for heart failure as well.
The risk of developing cardiotoxicity makes that careful patient selection for trastuzumab therapy is critically important. A reliable test to predict the occurrence of cardiac failure is therefore needed. In addition, there is no non-invasive method to visualize HER2 overexpressing tumor localizations. Radiolabeled trastuzumab might be useful for the detection of tumor localizations and also for the selection of those patients that should not be treated with trastuzumab, because they are likely to develop cardiac toxicity.

In **chapter 4**, the pre-clinical characterization of the development of radiolabeled trastuzumab is described. For the coupling of trastuzumab to ^{111}Indium (^{111}In), diethylenetriamine penta-acetic acid anhydride (DTPA) was used as a chelator. With size exclusion-high performance liquid chromatography, the radiochemical purity of the ^{111}In-DTPA-trastuzumab was determined and amounted to more than 95%. Furthermore, ^{111}In-DTPA-trastuzumab was stable in phosphate buffered saline over 14 days. The immunoreactive fraction, as determined by cell binding assays using human ovarian SK-OV-3 tumor cells, was 0.87. For an *in vivo* analysis of the biodistribution, athymic mice implanted with either a HER2

overexpressing (SK-OV-3) or a HER2 low expression (GLC4) human tumor cells were injected with indium-labeled trastuzumab. For the determination of tumor and normal tissue uptake of radiolabeled trastuzumab, the mice were sacrificed at 6 different time points ranging from 5 hours to 7 days. Tumor uptake in the mice implanted with the HER2-positive tumor was already present 5 hours after injection of the tracer, but was even more pronounced after 3 days. Uptake in organs such as the liver, spleen and kidneys was also visualized. Radiolabeling trastuzumab with ^{111}In, using DTPA as a chelator resulted in a highly stable tracer, which selectively binds to the human HER2 receptor both *in vitro* and *in-vivo* in animals. Based on these findings, ^{111}In-DTPA-trastuzumab was considered to be suitable for clinical use.

Following the pre-clinical validation of ^{111}In-DTPA-trastuzumab**,** the results of the clinical study with ^{111}In-DTPA-trastuzumab scintigraphy are presented in **chapter 5**. We hypothesized that the cardiotoxic and antitumor effects of trastuzumab might be specifically related to direct targeting and inhibition of HER2 expressed on cardiomyocytes or tumor cells, respectively. The primary aim of this study was to evaluate whether the radiolabeled trastuzumab scan could identify patients prone for developing trastuzumab-related cardiac dysfunction. As the secondary aim, we determined whether radiolabeled trastuzumab could be used to identify (HER2-positive) tumor lesions. Patients with HER2-positive metastatic or recurrent breast cancer were eligible for this study if they were suitable for treatment with trastuzumab and paclitaxel, and had a good cardiac function (radionuclide angiography) and performance score. Trastuzumab was administered weekly starting with the 4 mg/kg loading dose, followed by the maintenance dose of 2 mg/kg. Paclitaxel was given every 3 weeks at a dose of 175 mg/m^2. A radiolabeled trastuzumab scan was performed at the start of treatment and after 4 cycles. Patients underwent gammacamera imaging from 15 minutes to 7 days after tracer injection. Cardiac evaluations, consisting of a history and physical examination with specific attention to signs and symptoms related to the cardiovascular system, left ventricular ejection fraction (LVEF) measurement by radionuclide angiography and a cardiac ultrasound, were performed before the start of treatment, after 4 and after 6 cycles. Tumor evaluations were carried out every 2 treatment cycles. 17 patients were enrolled in the study, of whom 15 were evaluable. Pre-treatment myocardial uptake was observed in only one patient, who suffered from cardiac arrhythmias prior to enrollment in the study. Three other patients developed severe symptomatic heart failure during treatment, all without myocardial uptake on their pre-treatment scans. One of these three patients had myocardial uptake on the second scan after 4 cycles. Tumor lesions were visualized in most patients and in 13 patients lesions previously unidentified using routine staging examinations were visualized. Since none of the patients who developed cardiotoxicity during treatment had myocardial uptake on the pre-treatment scans, radiolabeled trastuzumab scintigraphy has limited value for predicting trastuzumab-related

cardiotoxicity, in the tested setting. However, the technique can be used to visualize HER2 positive tumor lesions and may assist in identifying metastases.

As described previously, the extracellular domain of the transmembrane HER2 can be proteolytically cleaved and shed into the circulation. The aim of the study presented in **chapter 6** was to evaluate whether serum HER2 levels are increased in patients with heart failure due to dilated cardiomyopathy or ischemic heart disease. Serum HER2 levels and plasma soluble (s) apoptosis-related proteins (tumor necrosis factor (TNF)α and its receptors sTNF-R1 and sTNF-R2) were measured with enzyme-linked immunosorbent assays (ELISA) in 50 patients with chronic heart failure. 15 healthy individuals served as controls. Serum HER2 was higher in patients with heart failure, compared to controls. In a three group comparison, between controls, New York Heart Association (NYHA) functional class II and NYHA class III patients, we observed a positive association between the NYHA class and serum HER2. Serum HER2 levels were inversely correlated with left ventricular function, as was assessed by measuring LVEF. In addition, plasma sTNF-R1 and sTNF-R2 were higher in patients than in controls and correlated positively with serum HER2. Based on these results, HER2 appears to be involved in the pathophysiology of heart failure. HER2 may also play a role in cardiomyocyte apoptosis, as was pointed out by the positive association between serum HER2 and sTNF-R plasma levels.
Chapter 7 describes a study concerning the use of measuring natriuretic peptides for the detection of cardiotoxicity in anthracycline-treated breast cancer survivors, after long-term disease-free follow-up. N-terminal atrial natriuretic peptide (NT-ANP) and B-type natriuretic peptide (BNP) were measured cross-sectionally after a median follow-up of 2.7 years and after more than 6 years following a relatively low dose of epirubicin (total dose either 360 or 450 mg/m^2) and chest wall irradiation. Plasma NT-ANP and BNP were measured with a radioimmunoassay in 54 patients, who had previously participated in two local trials evaluating cardiac dysfunction due to anticancer treatment. Median plasma BNP levels were increased during the follow-up period, while median NT-ANP levels did not change. Patients who had received 450 mg/m^2 epirubicin had higher BNP levels than patients treated with a total epirubicin dose of 360 mg/m^2. The findings of this study suggest that after the initial blow to the myocardium, an autonomically progressive process is initiated, which may lead to symptomatic cardiac dysfunction up to years after antineoplastic treatment. Although proper validation of these results are necessary, the simple use of plasma BNP measurement may become a valuable screening tool for the identification of patients at risk for developing cardiac dysfunction during the follow-up after anthracycline treatment.

Next to the more established natriuretic peptides as markers for cardiac dysfunction, we evaluated whether circulating apoptosis-related proteins can be used to detect (sub)clinical cardiotoxicity, during long-term follow-up after

adjuvant anthracyclines and radiotherapy for breast cancer. The secondary aim of this study, that is presented in **chapter 8**, was to determine whether the applied treatment regimens were of influence on the plasma apoptosis marker levels. With enzyme-linked immunosorbent assay (ELISA), we measured TNFα, sTNF-R1, sTNF-R2, sFas, sFas ligand, sTNF-related apoptosis-inducing ligand (sTRAIL) and serum HER2 in 34 breast cancer survivors, who had a median follow-up of 6 years after treatment with standard-dose 5 cycles of standard dose fluorouracil (500 mg/m^2), epirubicin (90 mg/m^2) and cyclophosphamide (500 mg/m^2) (FEC, n=14) or 4 cycles of FEC followed by high-dose myeloablative chemotherapy consisting of 1600 mg/m^2 carboplatin, 6 g/m^2 cyclophosphamide and 480 mg/m^2 thiotepa (FEC+CTC, n=20), followed by hematopoietic stem cell rescue. 12 healthy, age-matched women served as controls. NT-ANP, BNP, fibrinogen and high-sensitivity C-reactive protein were determined as markers for cardiac dysfunction. Circulating platinum was also determined in subjects who had received carboplatin in their myeloablative regimen. Chest wall irradiation and tamoxifen were started after recovery from chemotherapy. Although no associations between the circulating apoptosis markers and cardiac dysfunction was observed, higher levels were observed in breast cancer survivors compared to controls. sFas ligand and sTRAIL were higher in the high-dose than in the standard dose group. Circulating platinum concentrations were measured, but did not correlate with circulating apoptosis marker levels. The finding of higher apoptosis markers in breast cancer survivors than in healthy controls, particularly after high-dose chemotherapy, may be associated with late complications of treatment, such as cardiotoxicity, but require further study.

In **chapter 9**, the results of a prospective study are presented, evaluating circulating apoptosis marker levels in women during and after similar adjuvant breast cancer treatment regimens as described in chapter 8. The study was aimed at evaluating whether circulating soluble apoptosis-related protein levels change during the first year after the start of anthracycline-containing chemotherapy and radiotherapy in relation to cardiac dysfunction (primary endpoint) or to the applied treatment regimen (secondary endpoint). For this purpose, circulating tumor necrosis factor-related apoptotic proteins were determined in stored samples with ELISA in 40 breast cancer patients at three time points; prior to chemotherapy, one month after chemotherapy, and one year after the start of chemotherapy. Serum platinum levels were analyzed in subjects who had received carboplatin. We found that plasma apoptosis markers were not associated cardiac dysfunction, LVEF decrease, echocardiographic diastolic functional parameters, plasma natriuretic peptides or high-sensitivity C-reactive protein. One month after chemotherapy, sTNF-R1, sTNF-R2 and particularly sFas were transiently increased, whereas sFas ligand was transiently decreased, especially in the high-dose group. Circulating platinum levels one year after carboplatin-based chemotherapy were positively associated with plasma sTNF-R1 and sFas. Measurement of circulating apoptosis-related proteins

appears to have no value for the early detection of cardiotoxicity. Interestingly, we observed a transient change in circulating levels of these markers, particularly in high-dose chemotherapy-treated patients. Although transient, these changes may indicate that anticancer treatment has inflicted substantial damage, which might culminate into late complications of the treatment. To determine the (clinical) relevance of these findings, further study with long-term follow up will be required.

In **Chapter 10** a discussion and directions for future research are presented. Cardiac dysfunction is a worrisome side effect resulting from the use of several antineoplastic agents, of which the anthracyclines are the most well known. In addition, molecular targeted therapies with the potential of inducing cardiotoxicity, such as trastuzumab in the treatment of HER2-positive breast cancer patients, have been and will be used increasingly. Since the survival of patients treated for cancer has improved over the recent years, treatment-related morbidity and mortality become more relevant. Consequently, early detection, and preferably prevention, of anticancer treatment-induced cardiotoxicity becomes more and more important.