CHAPTER 01

GENERAL INTRODUCTION
Thoracic tumours such as breast, oesophageal, lung cancer and Hodgkin’s lymphoma are well known human malignancies. The treatment often includes radiation therapy. Survival rates of patients with different thoracic tumours varies widely from 5-year overall survival rates of approximately 80-90% for breast cancer and Hodgkin’s lymphoma patients to less than 25% for oesophageal and lung cancer patients.

Escalation of the radiation dose in lung cancer may lead to better locoregional tumour control and consequently overall survival. Unfortunately, the radiation dose that can be safely administered to the tumour is limited by the risk of radiation-induced toxicity of the surrounding lung and heart tissue. It has been predicted that a dose up to 100 Gy may be required to sterilize tumours of the size frequently treated in NSCLC. However, with the standard radiation dose of 60 Gy for lung tumours, even up to 20% of the patients develop symptoms and signs of radiation-induced lung toxicity. Symptomatic cardiac and pulmonary toxicity has been described in several retrospective studies and is clearly related to cardiac and/or lung dose distributions.

Despite recent improvements of radiotherapy to reduce radiation dose to the normal tissues with modalities such as Intensity-Modulated Radiation Therapy (IMRT), image-guided radiation therapy and proton therapy, symptoms and signs of radiation induced lung and cardiac toxicity still persists. More insight into the pathology of radiation-induced cardiac and pulmonary dysfunction (RICPD) could lead to strategies to diminish/ameliorate RICPD. This could improve radiotherapy treatment and even provide tumour dose escalation leading to a better tumour control and an increased survival of the patient.

Radiation-induced lung dysfunction (RILD) is a potentially life-threatening side effect of radiotherapy of thoracic tumours and was first described in 1898, soon after the development of X-ray images. The distinction between two separate types of RILD, an early inflammatory phase, termed “radiation pneumonitis” (RP), and a late fibroproductive phase, termed “radiation fibrosis”, was made in 1925. The early phase typically occurs around 6-12 weeks after radiotherapy and the late fibrotic phase months to years after treatment. Both pathologic processes may result in compromised lung perfusion, increased vascular resistance and reduced diffusion capacity. Clinical symptoms range from mild dyspnoea and a non-productive cough to respiratory failure requiring assisted ventilation, or even death.
Introduction

Radiotherapy for thoracic tumours

Thoracic tumours such as breast, oesophageal, lung cancer and Hodgkin’s lymphoma are well known human malignancies ¹. The treatment often includes radiation therapy. Survival rates of patients with different thoracic tumours varies widely from 5-year overall survival rates of approximately 80-90% for breast cancer and Hodgkin’s lymphoma patients ² to less than 25% for oesophageal and lung cancer patients ². Escalation of the radiation dose in lung cancer may lead to better locoregional tumour control and consequently overall survival ³,⁴. Unfortunately, the radiation dose that can be safely administered to the tumour is limited by the risk of radiation-induced toxicity of the surrounding lung and heart tissue. It has been predicted that a dose up to 100 Gy may be required to sterilize tumours of the size frequently treated in NSCLC ³,⁴. However, with the standard radiation dose of 60 Gy for lung tumours, even up to 20% of the patients develop symptoms and signs of radiation-induced lung toxicity ⁵,⁶. Symptomatic cardiac and pulmonary toxicity has been described in several retrospective studies and is clearly related to cardiac and/or lung dose distributions ⁷-⁹.

Despite recent improvements of radiotherapy to reduce radiation dose to the normal tissues with modalities such as Intensity-Modulated Radiation Therapy (IMRT), image-guided radiation therapy and proton therapy, symptoms and signs of radiation-induced lung and cardiac toxicity still persists ¹⁰-¹³. More insight into the pathology of radiation-induced cardiac and pulmonary dysfunction (RICPD) could lead to strategies to diminish/ameliorate RICPD. This could improve radiotherapy treatment and even provide tumour dose escalation leading to a better tumour control and an increased survival of the patient.

Radiation-induced cardiopulmonary dysfunction

Radiation-induced lung dysfunction
Radiation-induced lung dysfunction (RILD) is a potentially life-threatening side effect of radiotherapy of thoracic tumours ⁶ and was first described in 1898, soon after the development of X-ray images ¹⁴. The distinction between two separate types of RILD, an early inflammatory phase, termed “radiation pneumonitis” (RP), and a late fibroproductive phase, termed “radiation fibrosis”, was made in 1925 ¹⁵. The early phase typically occurs around 6-12 weeks after radiotherapy and the late fibrotic phase months to years after treatment. Both pathologic processes may result in compromised lung perfusion, increased vascular resistance and reduced diffusion capacity. Clinical symptoms range from mild dyspnoea and a non-productive cough to respiratory failure requiring assisted ventilation, or even death ⁶.
Pathology

Lung irradiation activates various cellular signalling pathways leading to the expression and activation of pro-inflammatory and pro-fibrotic cytokines, vascular injury, and the activation of a coagulation cascade. This consequentially results into the development of oedema, inflammatory responses, and the initiation of wound-healing processes \(^\text{16}\). Previous studies showed that a cascade of events on the cellular and molecular level starts immediately after irradiation, and proceeds during a period of clinically subclinical damage. Multiple factors in this process have been investigated, but the main initiating events and driving forces in the perpetuation of RILD are still largely unknown.

In patients, the pathologic and clinical changes in the lung following irradiation can be divided into 5 phases \(^\text{17}\).

The acute phase begins within hours to days after irradiation, and is generally subclinical. It is characterized by hyperaemic, congested mucosa with leukocyte infiltration and increased capillary permeability, leading to pulmonary edema. This is followed by an exudative alveolitis, accompanied by tracheal bronchial hypersecretion and degenerative changes in the alveolar epithelium and endothelium. Type I alveolar epithelial cells are sloughed, and levels of alveolar surfactant are increased \(^6\). During the next phase, the latent phase, thick secretions accumulate in the lung tissue due to increasing number of goblet cells combined with ciliary dysfunction.

The 3rd phase, the acute exudative phase, is clinically referred to as the “radiation pneumonitis” phase, occurring around 6-12 weeks after irradiation. It consists of sloughing of endothelial and epithelial cells, with narrowing of the pulmonary capillaries and microvascular thrombosis. Hyaline membranes are formed as a result of alveolar pneumocyte desquamation and leakage of a fibrin-rich exudate into the alveoli. Giant cells can be seen along the endothelium, and type II pneumocytes may become hyperplastic with marked atypia.

In the 4th phase, the intermediate phase, there may be resolution of the alveolar edudate and dissolution of the hyaline membranes, or there may be collagen deposition by fibroblasts, resulting in thickening of the interstitium. Fibroblasts, most likely of bone marrow origin, migrate into and proliferate within the alveolar walls and spaces \(^\text{18,19}\).

The final phase, clinically referred to as “radiation fibrosis”, consists of fibrosis. It may be evident as early as 6 months after irradiation, and can progress over years. The number of myofibroblasts within the interstitium and alveolar spaces is increased, along with an increase in collagen. The anatomic narrowing of alveolar spaces results in a decreased lung volume; vascular subintimal fibrosis and distortion cause a loss of capillaries. Eventually, traction bronchiectasis, complicated by chronic infections, can develop.

Prediction

Since RILD may lead to serious acute or late morbidity in patients after thoracic irradiation, the identification of factors predicting the risk of RILD is of major importance.

Many factors influence the development of RILD. These can be classified as treatment related factors (dosimetric parameters, concurrent chemotherapy), and patient related characteristics (pre-existing cardiopulmonary disease, smoking history, performance status etc.). All of these factors may have a certain prognostic significance in the development of RILD.
In fractionated radiotherapy, one of the most frequently-used dose-volume parameters to estimate the risk of developing RILD is the mean lung dose (MLD). Another frequently used method is to take into account specific dose-volume threshold parameters. However, controversy exists about which (dosimetric) parameter(s) optimally predict RILD. Concurrent rather than sequential chemotherapy appears to increase the risk of RILD, especially in women undergoing anthracycline-based adjuvant chemotherapy plus radiotherapy for breast cancer. In one report, the risk of early RILD in women treated with a supraclavicular field and concurrent versus sequential chemotherapy was 9 versus 1.3%, resp. Because of this risk, concurrent anthracycline-based chemotherapy and radiation are generally avoided in the treatment of breast cancer.

Furthermore, patient-related parameters may influence the development of RILD. Prior thoracic irradiation, volume loss due to lung collapse, younger age, smoking history, poor pre-treatment performance status, poor pre-treatment lung function, chronic obstructive disease, female sex, endocrine therapy for breast cancer, and glucocorticoid withdrawal during radiotherapy have all been reported to influence the risk of early RILD. Inclusion of these parameters may improve the performance of prediction models based on dosimetric parameters alone.

So far, only models predicting early RILD are described. Since improved treatment strategies may lead to a longer life expectancy of cancer patients, the occurrence of late radiation-induced normal tissue toxicity becomes more relevant. As such, to optimize radiation treatment, models predicting both early and late toxicity should be used.

**Radiation-induced heart dysfunction**

Radiation-induced heart dysfunction (RIHD) is generally believed to be a late side effect of radiation. The data on the late cardiovascular toxicity of radiotherapy comes primarily from survivors of Hodgkin’s lymphoma and breast cancer, diseases in which radiotherapy is a frequent component of the initial management and in which survival is often prolonged. However, radiation-induced cardiac toxicity has also been reported in lung and oesophageal cancer patients as described below.

A significant increase in the incidence of cardiovascular disease has been observed in multiple studies of long-term survivors of Hodgkin’s lymphoma (HL). The most common cardiovascular diagnoses were valvular disorders, angina pectoris, and myocardial infarction. The median time to diagnosis of these cardiac complications was approximately 19 years after treatment and persisted for at least 25 years after the initial treatment. Multiple studies have demonstrated that the risk of a fatal myocardial infarction in HL survivors is 2.2 to 7.6-fold greater than in the general population.

In breast cancer patients treated with postoperative radiotherapy, an increased risk of major coronary events for all time periods from the period less than 5 years through greater than 20 years was observed. The estimated mean dose of radiation to the heart was 4.9 Gy; the dose of radiation to the heart was higher in those with left-sided breast cancer (6.6 vs. 2.9 Gy). The risk of a coronary event increased progressively with the radiation dose, with an increase of 7.4% for each 1 Gy of radiation to the heart, with no threshold dose. A history of ischemic heart disease was associated with an increased excess risk of a cardiac event after treatment for breast cancer (ratio 6.67, p<0.001). Other factors also associated with a significantly increased risk included other circulatory disease and diabetes (risk ratios 1.88 and 3.23, respectively). However, the absolute increase in risk of a...
major coronary event or death form ischemic heart disease is small. Awareness of the potential cardiotoxicity of radiotherapy in breast cancer patients led to the application of improved radiation techniques that minimize irradiation to the heart. Unfortunately, even with the most advanced photon techniques such as IMRT and breath hold techniques, most women still receive doses of greater than 1-5 Gy to the heart. Contrary to breast cancer and HL only limited data is available on cardiac morbidity and mortality following radiotherapy of oesophageal cancer. This may be explained by the fact that cardiac toxicity has traditionally been regarded as a late side effect and the relatively low incidence of oesophageal cancer and previously low cure rates after treatment. Management of patients with locoregional oesophageal cancer generally includes radiotherapy or chemoradiotherapy. Because of the proximity of the oesophagus to the heart, cardiac exposure is unavoidable and can result in high doses of radiation being administered to the heart and pericardium. A recent review on clinically relevant cardiac complications after multimodality treatment for oesophageal cancer reported an overall crude incidence of 10.8% (range: 5-44%). Most events occurred within 2 years after radiation treatment. Pericardial effusion was the most frequently observed complication, with an actuarial rate of 48% \(^{37-40}\). Secondary ischemic events and heart failure were also frequently observed complications, however (chemo)radiotherapy may not have been the only risk factor for these cardiac events. Oesophageal cancer patients generally have a number of risk factors for heart disease. Multivariable prediction models are needed to investigate the influence of radiotherapy on the development of these cardiac events. Radiotherapy-induced cardiac toxicity in lung cancer patients has not been considered to be too clinically relevant. However, there are some data suggesting that meaningful cardiac damage is occurring in patients irradiated for lung cancer. The RTOG investigated the role of radiation dose-escalation in stage III NSCLC patients receiving concurrent chemotherapy, randomizing patients to receive 60 or 74 Gy \(^{41}\). Overall survival was worse in the high dose arm, with the curves separating within 6 months after radiotherapy. On multivariate analysis, the volume of the heart receiving ≥ 5 Gy and ≥ 30 Gy were independent predictors for overall survival, suggesting radiotherapy-related cardiac disease as a potential cause for the worsen survival rates as observed in the high-dose arm. Unfortunately, cardiac-specific toxicities were not assessed in this trial. These data may suggest that radiotherapy-associated cardiopulmonary injury in lung cancer patients can occur relatively soon after radiation.

Pathology

More insight into the pathology of radiation-induced cardiac damage may lead to strategies to diminish the damage. From previous studies it is known that irradiation of a substantial volume of the heart to a sufficiently high dose can damage virtually any component of a patient’s heart: the pericardium, myocardium, heart valves, coronary arteries, capillaries and the conducting system. \(^{42-44}\). A typical acute manifestation of radiation-induced heart toxicity is pericarditis. Chronic pericardial disease, coronary artery disease (CAD), cardiomyopathy, valvular disease and conduction changes can develop years after radiation treatment. \(^{26,32,45}\). Usually, pericarditis is self-limiting, however, 10-20% of patients develop a chronic or constrictive pericarditis 5-10 years post-irradiation. \(^{46}\). Associated pericardial effusions are recognized by fibrous adhesions, a high protein count, and an increase in inflammatory markers. Similar changes
are seen in mouse models of heart irradiation. Oedematous thickening of the pericardium, with inflammatory cell deposits and haemorrhage were observed after irradiation with a single dose of 16 Gy. Historically, pericarditis was the most common manifestation of radiation-induced heart toxicity. Fortunately, since modern techniques restrict the heart dose it is now rarely seen.

The musculature of the heart is relatively radio-resistant due to the lack of myocyte cell divisions. However, diffuse interstitial fibrosis develops after rather low radiation doses due to micro-vascular insufficiency and ischemia, involving damage to the capillary endothelial cells (ECs). There is experimental evidence that mast cells mediate collagen deposition in irradiated rat hearts. Moreover, radiation precipitates senescent changes in fibroblasts at doses as low as 1 Gy.

Diffuse interstitial fibrosis changes the compliance of the myocardium, leading to both systolic and diastolic dysfunction. Most patients with radiation-induced myocardial damage have some degree of interstitial fibrosis. This typically develops at radiation doses beyond 30 Gy. It has been shown that cardiac diastolic dysfunction is 7 times more common in patients treated with thoracic radiotherapy compared with control subjects.

Radiotherapy might directly damage the cardiac valves, leading to fibrotic thickening, valvular retraction and late calcification. The incidence of valvular dysfunction has been reported to increase during the 2nd decade after mediastinal radiotherapy for Hodgkin’s Lymphoma.

Next, radiation can damage the coronary arteries and capillaries of the heart. Initial endothelial cell (EC) damage/dysfunction is believed to be a key factor in the development of radiation-induced toxicity in the gut, and may also play a role in radiation-induced cardiac damage. Pathologically, radiation-induced vascular damage is accompanied by an increase in capillary wall permeability, generation of Reactive oxygen Species (ROS) and activation of inflammatory responses. These processes lead to intimal proliferation with collagen deposition and fibrosis. In vitro studies have also revealed the pro-thrombotic effect of radiation associated with higher release of von Willebrand factor which can provoke and contribute to coronary thrombosis.

Experimental studies suggest that radiation-induced atherosclerosis plays a role in the increased risk for myocardial infarction after high doses of radiotherapy to the thorax. The mechanism involved in plaque formation is thought to resemble spontaneous atherosclerosis; however, plaques in irradiated patients have been found to be more fibrous with a decreased lipid component.

Finally, abnormalities of the cardiac conduction system have been described after thoracic radiotherapy e.g. variable degrees of an atrial-ventricular block, sick sinus syndrome, arrhythmias, and ventricular tachycardia. Right bundle branch block is more often observed than left bundle branch block after irradiation, most likely due to a higher radiation dose to the right ventricle because of its anterior location. Ventricular ectopy might occur in up to 50% of patients exposed to mediastinal irradiation, often secondary to ventricular fibrosis. Dysfunction of the autonomic nervous system, leading to persistent tachycardia, loss of circadian heart rhythm, and respiratory phasic heart rate variability has also been described after irradiation.
Interaction between radiation-induced lung and heart dysfunction
In thoracic irradiation, parts of the lungs and heart are co-irradiated. Both preclinical and clinical studies showed an interaction between heart and lung damage after irradiation as described below.

In a study published by van Luijk et al. the effect of the heart on regional differences in lung function damage after irradiation was assessed in rats. Different dose distributions, either in- or excluding the heart were delivered to the rat’s thorax. RILD was assessed by BR measurements until 12 weeks post-irradiation. Irradiation of only the heart resulted in a low response similar to whole mediastinal region irradiation including the heart. Irradiation of a similar volume of lateral lung tissue alone, excluding the heart, resulted in a larger BR increase. When, however, the heart was co-irradiated with lateral parts of the lung (same lung volume with even slightly less alveolar tissue), a much more pronounced response was seen compared with the lateral field in which the heart was spared. These data were the first to show that co-irradiation of the heart had a strong effect on the manifestation of RILD early after irradiation. In a previous study, it was already shown that (co-)irradiation of the heart resulted in late RILD (>38 weeks after irradiation). This coincided with development of late RIHD, similar to the situation in humans. Early effects of heart irradiation on lung function had not been reported before.

The group of Nalbantov et al. investigated the association between pre-treatment cardiac comorbidity and the development of RILD in lung cancer patients after definitive radio(chemo)therapy. Interestingly, they found that cardiac comorbidity was an important risk factor for the development of RILD. The odds ratio of developing RILD for patients with cardiac comorbidity was 2.58 (p<0.01).

As such, these preclinical and clinical data suggest an interaction between lung and heart irradiation in the development of RILD. The mechanism of this possible interaction is not clear. Nevertheless, these data indicate that both the heart and the lungs may contribute to the development of early RILD. As such, for treatment optimization both heart and lung parameters may be taken into account.

Intervention strategies to reduce radiation-induced cardiopulmonary dysfunction

Intervention strategies to reduce radiation-induced cardiopulmonary dysfunction (RICPD) may provide tumour dose escalation leading to a better tumour control and potentially enhance the patient’s survival. Two potential strategies to ameliorate radiation-induced cardiopulmonary dysfunction are described below.

ACE inhibition
Until the mid-90s, radiation-induced normal-tissue toxicity was assumed to be solely caused by a delayed mitotic cell death. Then, since the mid-90s a shift occurred and it was discovered that cells do not only die but that they are also active participants in the response to radiation injury. This view gave opportunities for pharmacologic mitigation and/or treatment of radiation-induced damage. Angiotensin Converting Enzyme Inhibitors (ACEi) were one of the drugs studied in mitigating radiation toxicity.
The Angiotensin Converting Enzyme (ACE) is part of the Renin-Angiotensin (RAS) system. The RAS system is involved in regulating the blood pressure and water balance (Figure 1). A decrease of blood pressure/renal perfusion stimulates production of Renin by the kidneys. Renin can convert Angiotensin (AT), produced by the liver, to Angiotensin I (ATI). ATI can be converted by ACE, mainly produced in the lungs, to ATII. ATII can induce several actions by activating the AT receptor leading to an increased blood pressure such as sympathetic stimulation, water retention in the kidneys, arterial vasoconstriction and secretion of Anti Diuretic Hormone (ADH) leading to water absorption.

![RAS system diagram](image)

**Figure 1**: The Renin-Angiotensin (RAS) system.

Generally, ACEi are commonly used drugs to treat patients with cardiovascular diseases. Interestingly, preclinical studies indicated that suppression of the RAS may ameliorate radiation-induced toxicity in different organs like the kidneys, the central nervous system and also lungs.

In a study by Ghosh et al., rats were irradiated with a single dose of photons (10, 12 or 15 Gy) to the thorax. The ACEi Captopril or the AT receptor blocker Losartan was administered after irradiation. At 8 weeks after irradiation, Captopril improved survival and reduced radiation-induced increases in BR, changes in vascular reactivity, and histopathologic evidence of injury. Radiation-induced BR increases were even prevented if Captopril was started 1 week after irradiation or if it was discontinued after 5 weeks. Losartan, although effective in reducing mortality, was not as effective as Captopril in mitigating radiation-induced BR increase or altered vasoreactivity.

So far, the mechanisms of the protective effect of ACEi on radiation-induced toxicity are not clear. The study by Ghosh et al. suggests that mitigation of lung injury by captopril is not mediated solely by suppression of ATII activity by way of the ATI receptor, since Captopril (which decreases the generation of ATII) was more effective than Losartan, which blocks the response of ATII on the ATI receptor.

Apart from their hypotensive action, ACEi are known to have other properties such as an anti-inflammatory action. Further, it has been suggested that the sulfhydryl group in the
molecular structure of captopril confers in a free radical scavenger activity. All these effects can account in part for its radioprotection. Besides, it might act as an antioxidant to reduce inflammatory reactive oxygen species and thus mitigate radiation-induced toxicity. As such, ACEi seems to be an alluring strategy for attenuating RICPD. The interaction with cardiac damage after whole thorax irradiation has not been assessed before. Therefore, the effect of ACEi on early radiation-induced heart damage and the consequential effect on the development of RILD could be studied. Reduction of RICPD by ACEi may provide tumour dose escalation leading to a better tumour control and potentially enhance the patient’s survival.

Cell-based therapies

Stem cell therapies are increasingly tested to regenerate damaged normal tissue. As radiation sterilizes stem cells, the replacement of these cells by donor stem cells may prevent damage or restore function. As such, clinical protocols are currently being developed for the regeneration after radiotherapy of the salivary gland and the brain advocating the use of cellular therapies for other tissues. Adult tissue stem cells are responsible for the homeostasis of a tissue and the regeneration after damage. Therefore, cell replacement therapy for radiation-induced cardiopulmonary complications could be considered with various different cell types. For the heart, stem cell therapy is the best studied so far. Although cardiac stem/progenitor cells and even cardiomyocytes can be readily cultured in vitro, transplantation in the heart does not yet seem to lead to sufficient functionally integrated and long-term survival of cardiac stem cells.

For the lung, however, this may be a different story. In adulthood, the lung has reached a steady state with low cell turnover, but all lung regions can recover after injury. The lung consists of trachea, bronchioles and alveoli, each with a specialized role in respiration and host-defence. Several stem cells have been found which are responsible for the maintenance of specific parts of the lung. Kim et al characterized the bronchoalveolar stem cells that in response to cell injury give rise to Clara cells, the progenitors of ciliated epithelial cells, and AT2 cells. Recently, however, it was suggested that the trachea, bronchioles and alveoli are maintained by distinct populations. Clara cells, positive for Secretoglobin1a1 (Scgb1a1+) contributed to tracheal repair but not of other compartments. Basal cells are suggested to be a population responsible for the renewal of luminal cells. Interestingly, endogenous epithelial progenitor cells have been found in the adult lung and seem to be involved in the regeneration of airways, bronchioles and alveoli. Transplantation of several cell types has been shown to result in the formation of lung tissue in mice after damage. Among these are cultured pulmonary prominin-1+ epithelial progenitor cells (PEP), p63 expressing Krt5+ cells 60 and human lung derived c-Kit+ cells.

Under homeostatic conditions bone marrow derived non-hematopoietic stem cells, such as endothelial progenitor cells (EPCs), fibrocytes and mesenchymal stem cells (MSCs) circulate in the blood and contribute to repair of tissues such as lung in response to damage. Circulating EPCs are especially thought to be involved in the repair and regeneration of damaged blood vessels throughout the body. Two distinct subsets of EPCs can be derived from the bone marrow: the early outgrowth EPC and the late outgrowth EPCs, of which the
former mostly promotes angiogenesis through paracrine effects and the latter form vessels, albeit in a disease and tissue dependent manner. MSCs are multipotent stromal cells that can be isolated from several human tissues and are suggested to have the capability to differentiate into various cell types, such as bone, cartilage and also endothelial cells and may even act as immune-suppressants.

In summary, cell therapies for cardiopulmonary disease as described above have been shown, but not yet after irradiation. Therefore, these (stem) cell populations could be studied for their potential to support the regeneration of the cardiopulmonary system to enhance the therapeutic benefit of (chemo-)radiotherapy and to reduce the burden of side effects to patients and potentially enhance survival.

Aim and outline of the thesis

Improved prevention, intervention and prediction of radiation-induced cardiopulmonary dysfunction (RICPD) requires more knowledge into its underlying pathophysiology.

In the present thesis first we investigated the underlying pathophysiological changes in the cardiopulmonary system and their interaction on manifestation of dysfunction early after irradiation (Chapter 2 and 3). Here we discovered the important role of radiation-induced vascular damage in the development of early RICPD. This finding led us to our next study where we investigated if ACE-inhibitors, well known protectors of the cardiovascular system, could ameliorate RICPD (Chapter 4).

Since endothelial cell (EC) loss is a hallmark/initiating factor in the development of vascular remodelling leading to pulmonary hypertension, we investigated the role of EC loss in the development of RICPD. Furthermore, we investigated the role of pulmonary EC loss in the development of early RILD/RICPD and investigated if cell therapy as an intervention strategy (Chapter 5).

Since our studies thus far showed an important role of vascular damage in the development of early RICPD in a volume dependent manner, in the last chapter we investigated the role of vascular damage in late RILD/RICPD and the dose/volume dependency (Chapter 6).

**Chapter 2:** Previous studies performed in our lab showed that pulmonary vascular damage occurred after low doses early after irradiation, but the influence on the development of RICPD was not clear. Therefore, in this chapter we investigated the role of pulmonary vascular damage in the development of RICPD. Using our rat model with high-precision proton radiation beams and by irradiating small, intermediate and large volumes of rat lungs with graded radiation doses; we could dissect to what extent different levels of vascular damage were responsible for the cardiopulmonary dysfunction.

**Chapter 3:** From research in the cardiopulmonary field it is known that damage in either the lung or the heart may elicit secondary damage in the other. In the radiation field however, the interaction between these two organs in response to thoracic irradiation has not been shown. Our previous preclinical studies showed evidence of this interaction, albeit through unknown mechanisms. We therefore focused on unravelling the pathophysiology of this interaction in this chapter. Here again our rat model with proton irradiation was a suitable tool for elucidating the individual contributions of heart and lung
irradiation on tissue damage and cardiopulmonary dysfunction through the irradiation of each of them separately and determining the mutual response when both were irradiated.

**Chapter 4:** In this chapter we searched for an intervention strategy to ameliorate early RICPD. Since our studies thus far showed an important role of vascular damage in the development of RICPD inhibition of the renin-angiotensin system (RAS) seemed to be an alluring strategy. FDA approved ACE-inhibitors are known protectors of the cardiovascular system. Besides, preclinical studies indicated that suppression of the RAS may ameliorate radiation-induced toxicity in different organs like the kidneys, the central nervous system and lungs although the exact mechanism was not clear. Besides, in the latter study interaction with cardiac damage after whole thorax irradiation was not assessed. Using our rat model, we were able to irradiate either the lungs, heart or both and to investigate the effect of ACE inhibitors on early radiation-induced lung and/or cardiac dysfunction.

**Chapter 5:** It seems that ECs are an important target of radiation in many organs and may have a pivotal role in the development of early radiation-induced cardiopulmonary morbidity. Therefore, by using our rat model, we studied the role of endothelial cell loss in the development of radiation-induced (cardio)pulmonary dysfunction. Considering the pivotal role for EC loss, a cell-based therapy using EPCs may be an attractive option in the treatment of RILD. Therefore, in this chapter we investigated the potential of cell therapy to ameliorate early radiation-induced cardiopulmonary dysfunction by transplanting irradiated animals with EPCs followed by assessments of their cardiopulmonary function.

**Chapter 6:** The exact relationship between early and late RILD is still unknown. Technological developments in radiation therapy result in smaller irradiated volumes of normal tissue. Because the risk of radiation therapy-induced toxicity generally depends on irradiated volume, changing volume could change the dose-limiting toxicity of a treatment. Therefore, by using our rat model we investigated the dose-volume relationship of late RILD, assessed its dependence on early and late pathologies and studied if decreasing irradiated volume changed the dose-limiting toxicity.
Cirradiated volume changed the dose-limiting toxicity. Therefore, by using our rat model we investigated the dose-volume relationship of late irradiated volume, changing volume could change the dose-limiting toxicity of a treatment. Technological developments in radiation therapy result in smaller irradiated volumes of cardiopulmonary function. Dysfunction by transplanting irradiated animals with EPCs followed by assessments of their potential of cell therapy to ameliorate early radiation-induced cardiopulmonary attractiveness option in the treatment of RILD. Therefore, in this chapter we investigated the Considering the pivotal role for EC loss, a cell-based therapy using EPCs may be an advantageous strategy. FDA approved ACE-inhibitors are known protectors of the cardiovascular morbidity. Therefore, by using our rat model, we studied the role of endothelial cell loss in the development of radiation-induced (cardio)pulmonary dysfunction.

C: It seems that ECs are an important target of radiation in many organs and may be important as irradiated lung. The exact relationship between early and late RILD is still unknown. As important as irradiated heart tissue is for RILD, irradiated lymphoma.

C: The exact relationship between early and late RILD is still unknown. As important as irradiated heart tissue is for RILD, irradiated lymphoma.

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