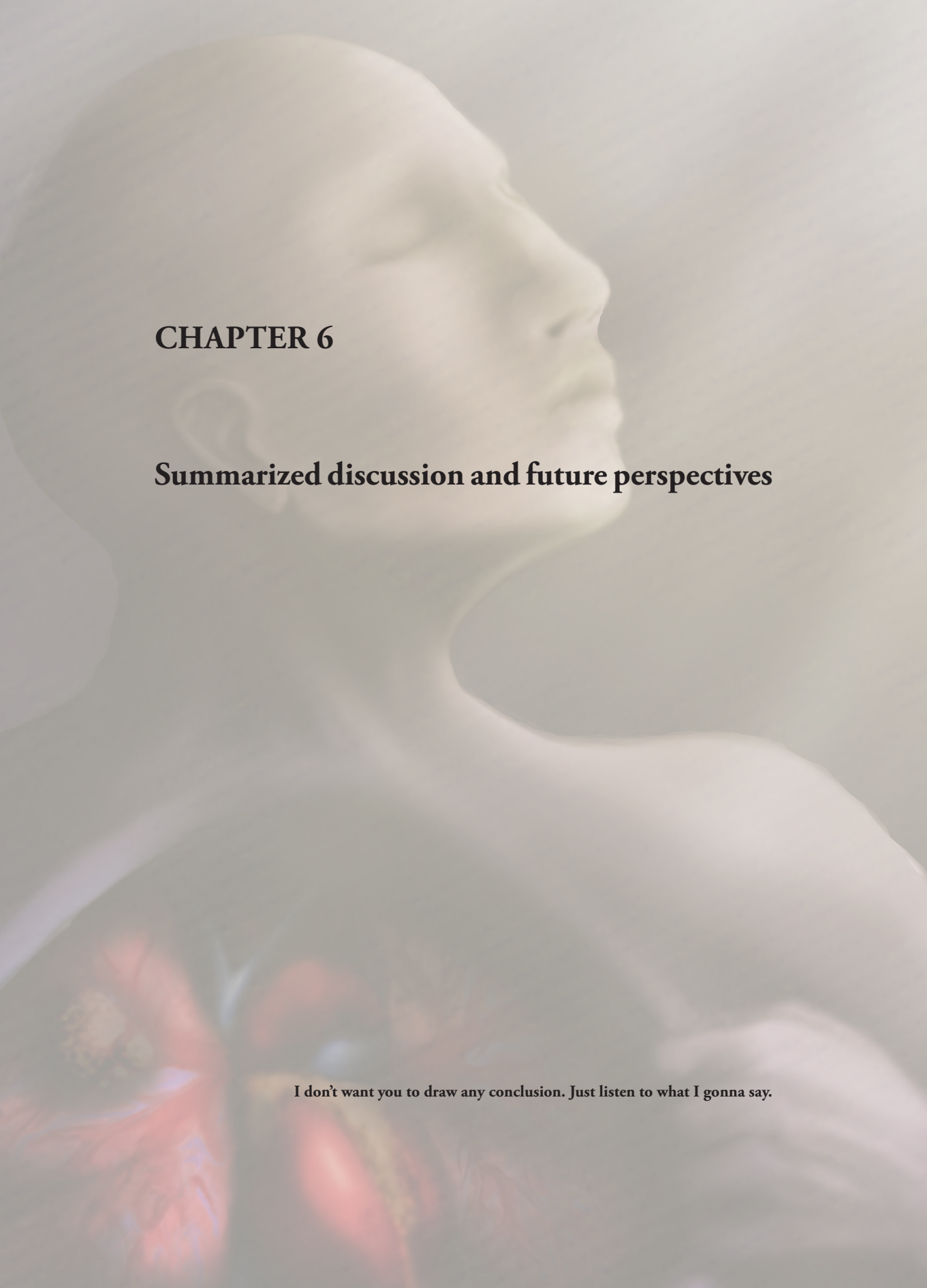
Pathophysiology of thoracic irradiation

Ghobadi, Ghazaleh



CHAPTER 6

Summarized discussion and future perspectives

I don't want you to draw any conclusion. Just listen to what I gonna say.

SUMMARIZED DISCUSSION

Radiation treatment plays an important role in the treatment of thoracic tumors [1,2]. The efficacy of the treatment however, is limited by the sensitivity of unavoidably co-irradiated healthy tissues [3-9]. In thoracic radiotherapy depending on the type, size and location of the tumor, parts of the healthy lung and/or heart are co-irradiated with the tumor causing toxicities [3-9]. One of the most common life-threatening complications after this treatment is thoracic radiation-induced respiratory dysfunction [3-8,10-13]. This complication is difficult to assess clinically since the criteria being used for its assessment are subjective and non-specific [4,10,25]. Respiratory dysfunction is often depicted as radiation pneumonitis and may subjectively experienced as some extent of breathlessness, "dyspnea", which is prevalent among lung cancer patients especially in locally advanced non-small cell lung carcinoma (NSCLC) occurring in up to a fifth of patients [8,14]. To develop a more efficient thoracic radiotherapy, a reduction of the chance of developing irradiation-induced respiratory dysfunction is needed. This can be achieved by accurate prediction of the risk, improved prevention and/or treatment of this complication. Attempts to develop methods for this have been made for several years, albeit with limited success due to insufficient insight into the fundamental processes leading to this complication [15,16]. Thoracic radiotherapy-induced dyspnea is mostly referred to as being caused by radiation pneumonitis, an early inflammatory reaction involving the lung parenchyma [10]. This consensus however, is challenged by recent preclinical and clinical findings [17-22]. Previous preclinical findings from our group pointed towards a critical role of the pulmonary vascular system and the heart in the development of thoracic irradiation-induced respiratory dysfunction [18,20]. Therefore, in chapter 2 and 3 we focused on these anatomical substrates in our rat model and showed that thoracic radiation-induced damage to the lung and the heart are very much inter-related and that respiratory dysfunction becomes manifest through physiological changes in the whole cardio-pulmonary system rather than through changes in the lung only (Figure.1).

Respiratory dysfunction in preclinical studies in the field of thoracic irradiation is measured by increases in breathing rate [19]. As mentioned earlier, it is generally accepted that subjective experience of dyspnea may reflect a reduced diffusion capacity due to

parenchymal damage in the lung [10]. The findings of chapter 2 and 3 showed however that physiological changes in the cardio-pulmonary system of the rats might be the main driving force of this response rather than parenchymal damage.

In Chapter 2 we described that lung irradiation increases pulmonary pressure leading to pulmonary hypertension in rats, in an irradiated-volume dependent manner. This may originate from perivascular edema and vascular remodeling in the whole lung, possibly initiated by global loss of pulmonary endothelial cells. Global loss of endothelial cells was observed prior to breathing rate increase and any morphological changes in the parenchyma, suggesting that vascular response may precede parenchymal damage. The increase in pulmonary pressure correlated strongly with the increase in breathing rate suggesting that lung irradiation-induced respiratory dysfunction in rats may be due to the vascular remodeling and not necessarily only by lung parenchymal damage.

In Chapter 3 it was described that radiation-induced pulmonary hypertension and pulmonary perivascular edema secondarily impair left ventricle diastolic function by increasing the relaxation time of the left ventricle. This may be caused from decreased blood input from the lungs back to the heart. Left ventricle diastolic dysfunction also occurs after heart irradiation, possibly initiated from radiation-induced cardiac perivascular fibrosis. This direct effect of irradiation of the heart on diastolic function induces a secondary effect on the lung by promoting pulmonary interstitial edema.

These findings clearly show that heart and lung irradiation can cause independently both cardiac and pulmonary damage/dysfunction, which secondarily affect each other through their vascular connections (Figure.1). Furthermore, simultaneous irradiation of both organs combined these effects and subsequently enhanced respiratory dysfunctio measured as pronounced breathing rate increase in the rats. Here both right and left ventricles of the heart are compromised and the combination of lung irradiation-induced perivascular edema and heart irradiation-induced interstitial edema causes excessive accumulation of fluid in the pleura, which may be the determinant of enhanced respiratory dysfunction. Cardiac diastolic dysfunction, pulmonary hypertension and the resultant pulmonary edema and pleural effusion are all known to impair ventilation by reducing perfusion due to either backward failure of the LV or vascular damage, causing

congestion of the pulmonary vasculature [23,24].

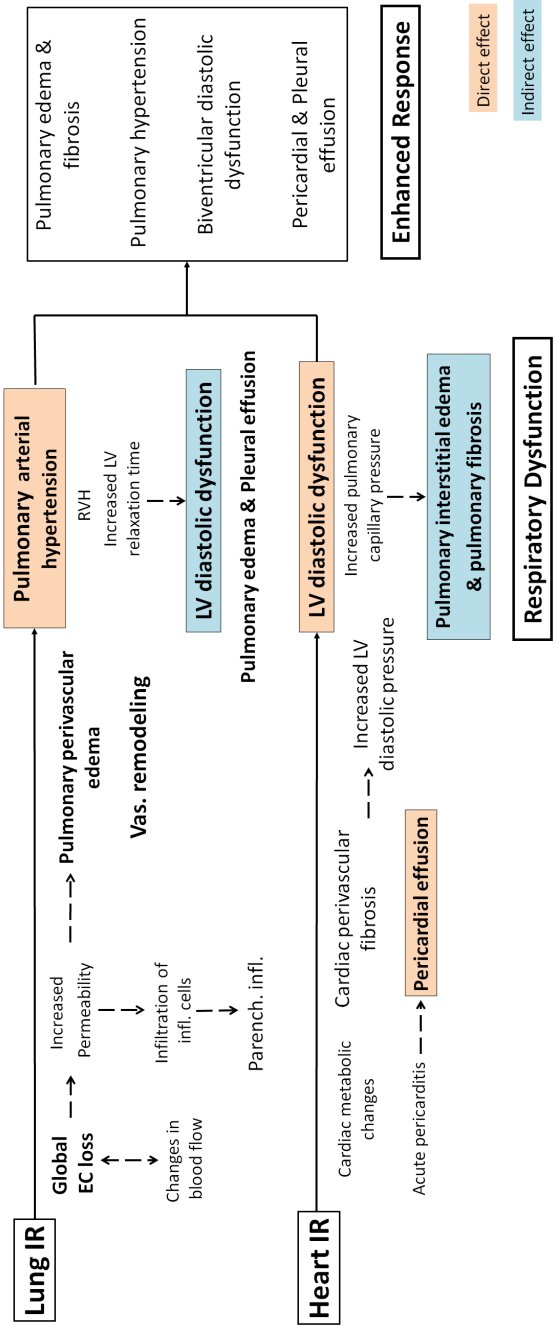


Figure.1: Pathophysiology of thoracic radiation-induced cardio-pulmonary toxicity in the rats. Lung and heart irradiation independently induce changes in each other, which becomes aggravated by combined irradiation of both. IR: irradiation, EC: endothelial cell, LV: left ventricle, RVH: right ventricle hypertrophy, Vas.: vascular, Infl.: inflammation, Parench: parenchymal.

Clinically, in patients with these kinds of cardio-pulmonary diseases, the decreased ventilation efficiency manifests as respiratory dysfunction, which is subjectively experienced as dyspnea [23]. Similarly, the thoracic radiation-induced respiratory dysfunction in our preclinical setting may have originated from the described physiological changes in the cardio-pulmonary system rather than from the lung parenchymal damage only (Figure.1). This, however, needs testing in a clinical setting to show clinical relevance for patients after thoracic radiotherapy. In the clinical practice of radiotherapy however, as mentioned before, the criteria being used for the assessment of respiratory dysfunction are subjective and non-specific [4,10,25]. Use of objective quantitative parameters, such as Computed Tomography (CT) scans of the lungs may improve this assessment. As mentioned earlier thoracic radiation-induced injury to lung tissue in rats was found to be a global tissue response, possibly due to the role of cardio-pulmonary physiological changes. Even with clinical reports [26], this global tissue response has not received much attention yet since with routinely used methods it could not be confirmed in the clinic. This may be due to the lack of sensitivity of currently used methods. Therefore, to be able to translate our preclinical findings to the clinic we developed a more sensitive CT-based tool (ΔS method) to assess radiation-induced lung tissue damage (chapter 4 and 5). This method quantifies the structural changes in the lung tissue (ΔS) by including variation of the lung density in addition to routinely used mean lung density changes. The ΔS method showed to be superior to the previously used methods, being more sensitive and specific in detecting lung tissue damage and dyspnea in both preclinical (chapter 4) and clinical settings (chapter 5).

FUTURE PERSPECTIVES

1. FUTURE PERSPECTIVES IN THE THORACIC RADIOTHERAPY

As mentioned earlier, attempts to predict, prevent and intervene in thoracic irradiation-induced respiratory dysfunction have largely failed so far [15,16]. New considerations should be made by acquiring more insight in the pathophysiology of the processes leading to this complication. Preclinical studies offer this opportunity, since the effect of dosimetric parameters on pathophysiological processes can be tested in a well-controlled manner and over a wide range. Several of these studies including ours [18,20,27-33] indeed found new aspects and suggested contribution of other factors such as cardio-pulmonary physiological changes in the development of respiratory dysfunction. The presence of these phenomena in the clinic has yet to be shown and validated in patient populations with thoracic tumors. We cannot use the same tools in humans as we use in animals, therefore non-invasive tools such as echocardiography, MRI and CT are imperative for the assessment of the post-treatment changes in the lung and the heart. Thoracic radiotherapy-induced damage to the lung tissue is routinely assessed using CT-based tools [3,26,34], which untill now have not indicated changes in the structure of the lungs in the low dose regions (≤ 27-40 Gy). Furthermore, the assessed changes in the lungs were not shown to be exclusively associated with symptomatic patients nor were the symptoms limited to patients with these changes [26]. These together suggest that our observations in animals are not valid for patients or that the current methods may not be sufficiently sensitive. Indeed, our newly developed ΔS method was shown to be capable of detecting lung tissue damage in regions receiving negligible dose and be a more sensitive and specific tool in detecting symptomatic patients, therefore adding a superior tool to the routinely used CT-based tools (chapter 5). The results of the current work not only propose a new perspective in the pathophysiology of thoracic radiation-induced cardiopulmonary toxicity and the development of respiratory dysfunction and dyspnea, but also provide a more sensitive tool in the assessment of the manifestation of potentially related lung density changes. Based on our current findings, new methods for prevention and/or treatment of lung/heart toxicity as well as prediction of their risk may be developed.

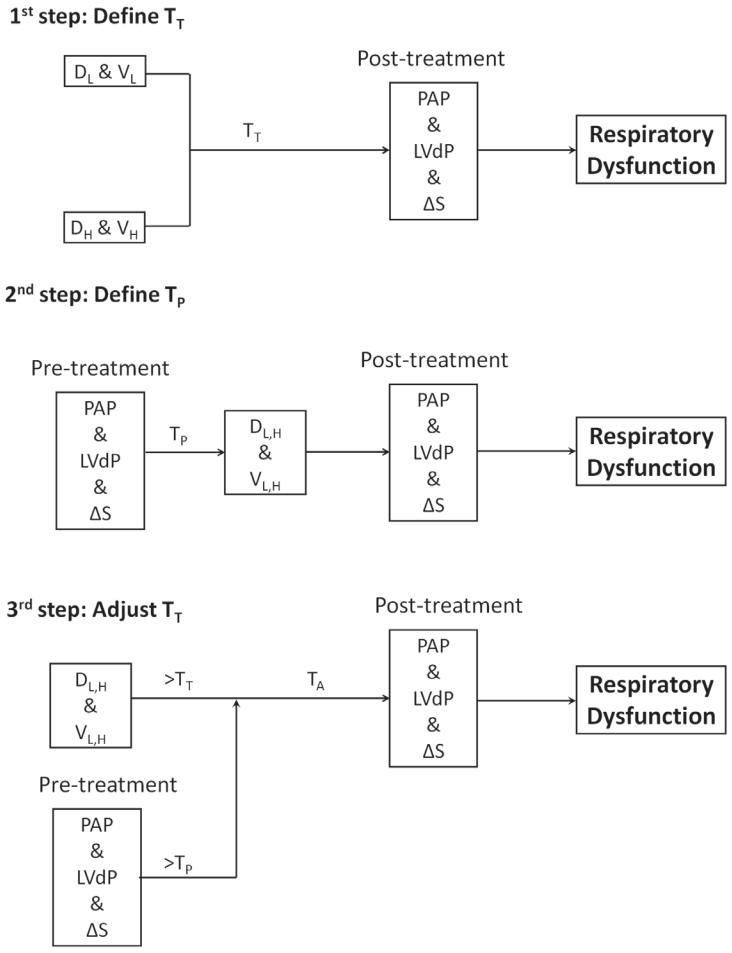


Figure.2: Biology-based prediction of the risk of cardio-pulmonary complications after thoracic irradiation in a preclinical setting. Incorporating additional factors such as dosimetric parameters of the heart, pre-treatment cardio-pulmonary parameters (e.g. PAP) and lung structure information (S) into predictive models for the risk of thoracic irradiation-induced cardio-pulmonary toxicity may lead to more accurate prediction. Pulmonary artery pressure (PAP), left ventricle diastolic pressure (LVdP) and lung structural changes (ΔS) can be indirectly assessed using noninvasive techniques such as echocardiography, MRI and CT scans. Establishing the relation between pre-treatment parameters and the post-treatment changes in different situations of thoracic irradiation (different DL,H and VL,H) in rats helps to determine an endpoint or endpoints to which dyspnea relate to the best in each situation.

In preclinical settings we have the opportunity to establish a comprehensive overview of the dose-volume relationships with cardio-pulmonary parameters by well-controlled induction of the effects using a wide range of dosimetric and volumetric parameters in the lungs and the heart. This helps to determine critical dosimetric and volumetric parameters with which respiratory dysfunction occurs in different situations of thoracic irradiation (Figure.2, first step). The effect of these dosimetric and volumetric parameters can be tested on individual post-treatment cardio-pulmonary endpoints such as pulmonary artery pressure (PAP), left ventricle diastolic pressure (LVdP) and S to determine which endpoint or endpoints irradiation-induced respiratory dysfunction correlates most closely to, in different situations of thoracic irradiation. Pre-existing cardio-pulmonary vascular disease may also affect the risk of the development of treatment-related cardio-pulmonary complications [22,35]. Therefore, pre-treatment physiological and structural parameters should be added to dosimetric and volumetric parameters and the effect of pre-existing levels of both on post-treatment changes should also be determined (Figure.2, second step). In a pre-clinical setting, pre-treatment cardio-pulmonary diseases can be induced to different levels by other means than irradiation, e.g. monocrotaline induction [36-38]. The effect of irradiation on these types of animal models should however be first established. This is an exercise for determination of the most relevant endpoints to the risk of irradiation-induced respiratory dysfunction in different situations of thoracic radiotherapy with different lung and heart dose-volume and pre-treatment parameters. Although these relations still should be tested and verified in clinical studies, this preclinical practice provides critical biological information needed to allow building of hypothetical predictive models of thoracic radiotherapy-induced respiratory dysfunction, which can/should be validated in the clinic. Many reports have attempted to identify potential risk factors and improve such predictive models [39-44], but due to insufficient knowledge in the biological processes leading to this complication, progress has been modest [8,15]. Based on our pre-clinical findings on the critical role of the heart [20,45], recently Huang EX et. al. [21] showed in a population of lung cancer patients improvement in the risk modeling of radiation pneumonitis as a surrogate for irradiation-induced respiratory dysfunction, by adding heart-related parameters to currently-used

lung-related ones such as mean lung dose [39-44]. Moreover, Jenkins P. et. al. [46] could also recently significantly improve the predictive models by incorporating physiologic parameters such as transfer factor for carbon monoxide into the dosimetric-related ones. They found that the geometric position of tumors, irrespective of dose–volume parameters, is important in the development of radiation pneumonitis [46], albeit without describing an underlying mechanism. Caudally situated tumors in the thorax between vertebra levels of T5-T9 were associated with higher risk [46]. The heart is located in this position in the thorax which could suggest that irradiation of the tumors around the heart are associated with higher risk of developing respiratory dysfunction.

Taken all together, we suggest that dosimetric parameters in the lungs may not be the only component determining respiratory dysfunction and other factors such as heart dosimetric/volumetric parameters (e.g. VH, DH), pre-existing cardio-pulmonary diseases (e.g. pre-treatment pulmonary artery pressure) (Figure.2) should also be taken into account for a more accurate prediction of the risk.

1.1 Individual-tailored thoracic radiotherapy

5-20% of the patients treated for thoracic tumors establish acute irradiation-induced clinical symptoms including dyspnoea [5]. Currently the severity of the toxicity is graded and the treatment given only after such symptoms have been noted [5]. More accurate prediction however, means that practically a radiation-oncologist can identify patients at risk based on, for example, patient's pre-existing diseases, the type, size and location of tumor in advance. Yet efficient thoracic radiotherapy will only be achieved when these individuals receive optimum prevention or treatment of the to be expected clinical symptoms. Since the clinical symptoms are believed to be caused by the inflammatory response in the lungs, the initial treatment of choice for the symptomatic patients is currently anti-inflammatory medicines e.g. corticosteroids [5,11,47]. Limited published evidence however, exists showing the efficacy of these medicines. Furthermore, there is a lack of pre-clinical and clinical data evaluating the outcomes of other therapies [5].

As mentioned earlier, based on our preclinical findings, lung parenchymal inflammation may not initiate the cascade of the events leading to respiratory dysfunction (Figure.1). Lung parenchymal inflammation could be a secondary effect originally initi-

ated from pulmonary vascular changes (e.g. activation of ECs) or cardiac damage, well known to mediate the influx of inflammatory cells in the lung [48-50]. Suppressing inflammation by corticosteroids may therefore only solve the resultant effects. This may explain why the symptoms recur after the cessation of corticosteroids therapy [3].

Instead of the general use of corticosteroids therapy for all symptomatic patients, other treatment strategies could potentially be envisioned in the future if our pre-clinical data are confirmed in the clinic. Such treatments could consist of an individual treatment in which optimal combinations of prevention/treatment strategies can be selected for each patient based on radiotherapy- and patient-related parameters (Figure.3).

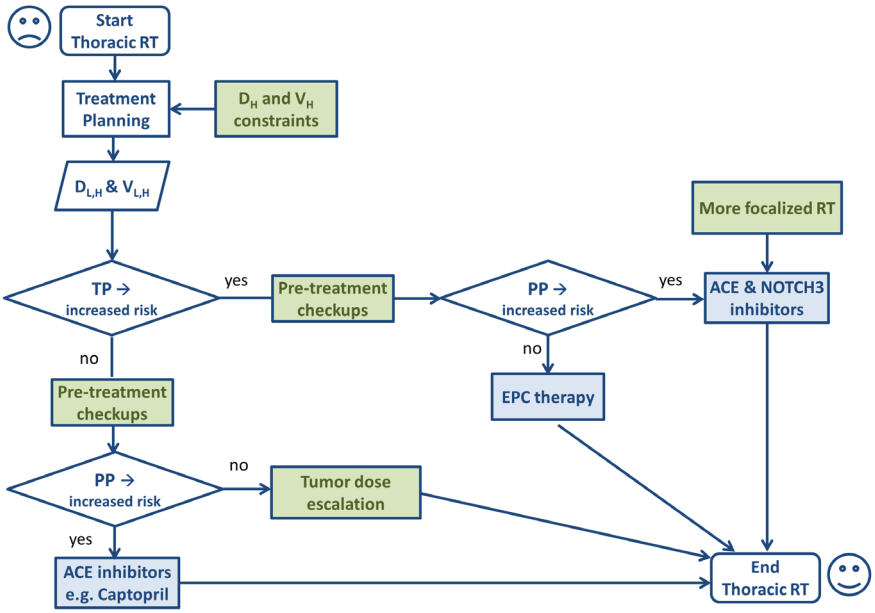


Figure.3: Potential individual-tailored thoracic radiotherapy. Blue boxes: Treatment strategies for thoracic radiotherapy-induced cardio-pulmonary complications; green boxes: Optimization of thoracic radiotherapy techniques. RT: Radiotherapy, DH: Heart irradiation dose, VH: Heart irradiated volume, DL,H: Lung and heart irradiation dose, VL,H: Lung and heart irradiated volume, TP: Treatment-related Parameters, PP: Patient-related Parameters, EPC: Endothelial Progenitor Cells, ACE: Angiotensin Converting Enzyme.

This, as mentioned earlier, will not be feasible without first testing different prevention/treatment strategies for different combinations of pre-treatment and treatment-related parameters in pre-clinical settings, and then validating them in the clinic. When a patient is referred to thoracic radiotherapy, first the dose-volume parameters for the lung and the heart (treatment-related parameters: TP) are generated at the treatment planning. Next to this, with routine pre-treatment checkups for each individual patient, the level of pre-treatment cardio-pulmonary parameters (PP) such as PAP, LVdP and S can be determined by e.g. CT scans and echocardiography (Figure.3).

Based on accurate biology-based predictive models, it can be predicted that whether these treatment and pre-treatment parameters (TP and PP) are associated with clinical symptoms (Figure.3). Depending on that, a treatment or combinations of treatments can be selected for patients whom are at risk to develop complications. Based on the preclinical findings of the current work, targeting changes in pulmonary vasculature (e.g. endothelial cell loss) and/or cardiac damage (e.g. perivascular cardiac fibrosis) may be potential treatment strategies for thoracic irradiation-induced respiratory dysfunction.

1.2 Endothelial progenitor cell therapy

Bone marrow derived stem cells have been shown pre-clinically to be capable to contribute to the regeneration of radiation-injured organs such as salivary glands [51,52]. Under homeostatic conditions bone marrow derived non-hematopoietic stem cells, such as endothelial progenitor cells (EPCs), fibrocytes and mesenchymals stem cells (MSCs) circulate in the blood and contribute to repair of the lung in response to damage [53]. Circulating EPCs are thought to be involved in the repairing and regeneration of damaged blood vessels throughout the body and have been shown to participate in re-endothelialization and neo-vascularization [54]. We showed in rats that lung irradiation induces endothelial cell loss, which might initiate the cascade of vascular remodeling, pulmonary hypertension and eventually respiratory dysfunction (chapter 2). Timely replacement of these cells using bone marrow derived EPCs may prevent this whole cascade. Such cell-based therapy recently showed potential success both pre-clinically and clinically in the demonstration of ability to attenuate monocrotaline/hypoxia-induced pulmonary hypertension in rats, dogs and mice [55,56] , as well as in pediatric and adult patients with idio-

pathic pulmonary arterial hypertension [57,58]. These findings warrant experimental studies to investigate their possible therapeutic effect after thoracic irradiation. When successful, it may open the road to the clinical use of these strategies in thoracic radiotherapy. Such a treatment may be useful in patients without pre-existing cardio-pulmonary diseases of which the tumor size necessitates irradiation of a large lung volume and potentially more severe pulmonary hypertension (Figure.3). The effect of such therapy on the tumor growth however first needs to be carefully investigated.

1.3 Intervening in vascular remodeling pathways

For patients with pre-existing cardio-pulmonary diseases, EPC therapy may not be efficient since the endothelium layer of their pulmonary vessels may already be remodeled[59-64]. Large population of patients undergoing thoracic radiotherapy, especially those with lung and esophagus cancers, present with these diseases prior to the radiation treatment [4,5,8,14]. This population may be more prone to develop thoracic radiotherapy-induced respiratory dysfunction since for instance pre-treatment pulmonary pressure is known to be a risk factor [22]. Depending on treatment- and patient-related parameters, different treatment strategies might be employed for them to ameliorate the extra stress that the thoracic irradiation may impose on their cardio-pulmonary system (Figure.3). For patients with pre-existing pulmonary vascular disease such as pulmonary hypertension, for whom treatment-related parameters (TP) correlates with an increased risk of respiratory dysfunction, treatment strategies, which target the vascular remodeling pathways, may be beneficial (Figure.3). Changes in endothelium and the resultant alteration in Nitric Oxide (NO) signaling [65] play a critical role in the development of vascular remodeling [66] which if inhibited, attenuates pulmonary hypertension [67]. Combination of this treatment with EPC therapy dramatically reduced monocrotaline-induced pulmonary hypertension in rats [55]. Thoracic irradiation also increases the expression of NO in the lung [68] which when attenuated led to decreased level of lung injury in rats [6]). Targeting NO signaling pathway alone or in combination with other therapies could therefore, be a promising treatment strategy. Another possible strategy may be targeting the NOTCH3 pathway, which is well known to be central in controlling the proliferation of vascular smooth muscle cells in small pulmonary arteries, one of the main features of pulmonary

vascular remodeling [70,71]. Inhibition of NOTCH3 pathway effectively prevented hypoxia-induced pulmonary hypertension in mice [70].

1.4 Treatment for cardiac dysfunction

The presence of clinical/sub-clinical cardiac damage in our animals models might point towards a role for this in the development of thoracic irradiation-induced respiratory dysfunction and dyspnea in patients (chapter 3). When this is the case, treatment strategies targeting such damage may also be needed for a more efficient treatment. This could be beneficial for patients who present with cardiac diseases e.g. cardiac diastolic failure prior to thoracic radiotherapy (Figure.3). Current practice in the optimization of the treatment of thoracic tumors such as NSCLC does not take the dose distribution in the heart in the relation to acute respiratory complications into account [8]. We however, showed in rats that for a given lung dose, increasing dose to the heart might in fact enhance this risk (chapter 3). This could suggest that adding dose constraints for the heart might further optimize thoracic radiotherapy particularly for patients of whom large volume of the lung has to be co-irradiated. Patients for whom the size and location of the tumor inevitably exposes their heart to irradiation could benefit from treatment strategies that ameliorate clinical/sub-clinical cardiac damage (Figure 6.3). Angiotensin II (Ag II) receptor antagonists or angiotensin converting enzyme (ACE) inhibitors are currently been used to treat patients with cardiovascular diseases [72]. Interestingly, the ACE inhibitor captopril has also been shown to reduce myocardial fibrosis after localized heart irradiation in rats [73]. Ag II is known to initiate cardiac perivascular fibrosis and diastolic dysfunction [74,75] and be involved in the induction of pulmonary fibrosis during cardiac dysfunction [49,76]. We observed that co-irradiation of the heart induces cardiac perivascular fibrosis and diastolic dysfunction as well as significant amount of pulmonary fibrosis in rats (chapter 3). Interestingly, in the clinic, acute respiratory complications and post-radiotherapy radiologic changes such as lung consolidation were less likely to develop in lung cancer patients who used ACE inhibitors [26,77]. Taken together, these observations suggest that modulation of angiotensin signaling may ameliorate cardiac fibrosis and diastolic dysfunction as well as pulmonary edema and fibrosis, and may be of a great therapeutic interest for the treatment of thoracic irradiation-induced acute respiratory dysfunction.

This therapy may be a good treatment option for patients of whom the size of tumor and proximity to the heart requires irradiation of large volume of the lung and the heart (Figure.3). These patients could potentially also be good candidates for more focalized radiation treatments such as particle therapy e.g. by applying proton beams (Figure.3). Because of the physical characteristic of proton beams, including steep dose gradient, they can be used for treatments in which organ preservation is very important [78]. For instance, in the treatment of unresectable NSCLC, protons may offer the opportunity to confine the irradiation to the target volume and avoid spreading low dose of irradiation to large volume of the healthy tissue. It was shown that in comparison to standard photon therapy, proton radiotherapy for lung cancer significantly reduces the dose to normal lungs and heart, even with dose escalation [79-81]. For these tumors however, uncertainties in proton treatment planning caused by tumor motion and changes in lung density due to respiration are still challenging [82].

As described above, the findings of the current work suggest to study new considerations for optimization of thoracic radiotherapy, which after validation in the clinic may eventually change the current practice by introducing new avenues to develop not only interventions for prevention/treatment of the complications but also more accurate risk models for their prediction (Figure.3). Applying these strategies may improve the effectiveness of thoracic radiotherapy. For instance, in radiotherapy of NSCLC the patients with the lowest tolerance for developing acute respiratory complications due to for example pre-existing cardio-pulmonary disease can be accurately identified and excluded from the remaining population. Subsequently, this allows for tumor dose escalation in the residual population and thereby they may have a higher local tumor control. The identified population at risk could also experience less toxicity either after applying more effective treatment strategies combining radiotherapy with e.g. ACE inhibitors and/or optimized radiation treatment techniques with e.g. particle therapy. All the suggestions described might, when valid for patients lead to more efficient thoracic radiotherapy.

2. IMPLICATIONS TO THE FIELD OF CARDIOPULMONARY VASCULAR DISEASES

Despite improvement in therapeutic regimens, PAH still has a poor prognosis [62,83,84]. This is in part due to the lack of good experimental models, which can produce the characteristics of PAH such as neointima lesions [37]. Besides giving opportunities for improving thoracic radiation treatment, the findings described in this thesis introduce a new model that could be used for the study of underlying mechanisms of lung and heart interaction in the field of cardiopulmonary vascular diseases such as pulmonary arterial hypertension (PAH) and heart failure. The animal radiation model provides potential tools for inducing different levels and states of these diseases by tuning dosimetric and volumetric factors of lung and heart irradiation. Indeed, our lung irradiation model closely resembles PAH (chapter 2). Unique features of radiation model such as endothelial changes, presence of neointimal lesions both in and out of the irradiation field, well-controlled induction of damage and partial recovery in time make it a promising new model of PAH. Studying the pathophysiology of the development and recovery of the radiation model of PAH offers opportunities for developing prevention and treatment strategies of PAH. The combined irradiation of the lung and the heart produces severe right ventricle diastolic dysfunction in combination with PAH. Severe right ventricle dysfunction is a major determinant of outcome in PAH, and may strongly influence the clinical management. The general concept is that right ventricle failure associated with PAH is strictly due to the increased right ventricle afterload [85]. A new paradigm however, argues that chronic pulmonary artery pressure elevation is insufficient to explain right ventricle failure and right ventricle fibrosis is also required [85]. In this respect, the combined heart and lung irradiation in which right ventricle dysfunction occurs with PAH in the presence of right ventricle fibrosis (chapter 3) is a potential model to study the pathophysiology of right ventricle failure in general.

Taken together, the findings of the current work show for the first time that thoracic irradiation-induced respiratory dysfunction in rats is a multi-organ complication that develops through physiological changes in the whole cardio-pulmonary system, rather than through changes in the lung only. This, although still in preclinical stage,

suggests new considerations in the optimization of thoracic radiotherapy. These findings could be helpful with the development of new methods for more effective treatment of thoracic radiotherapy-induced dyspnea and more accurate prediction of its risk, potentially leading to a more individualized thoracic radiotherapy and improvement of quality of life for patients undergoing this therapy. Furthermore, studying the pathophysiology of thoracic radiation-induced cardio-pulmonary toxicities offers opportunity to study and develop strategies for the prevention and treatment of cardio-pulmonary vascular diseases in general such as pulmonary arterial hypertension.

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