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Cardiotoxicity induced by radiotherapy and/or chemotherapy after cancer treatment.

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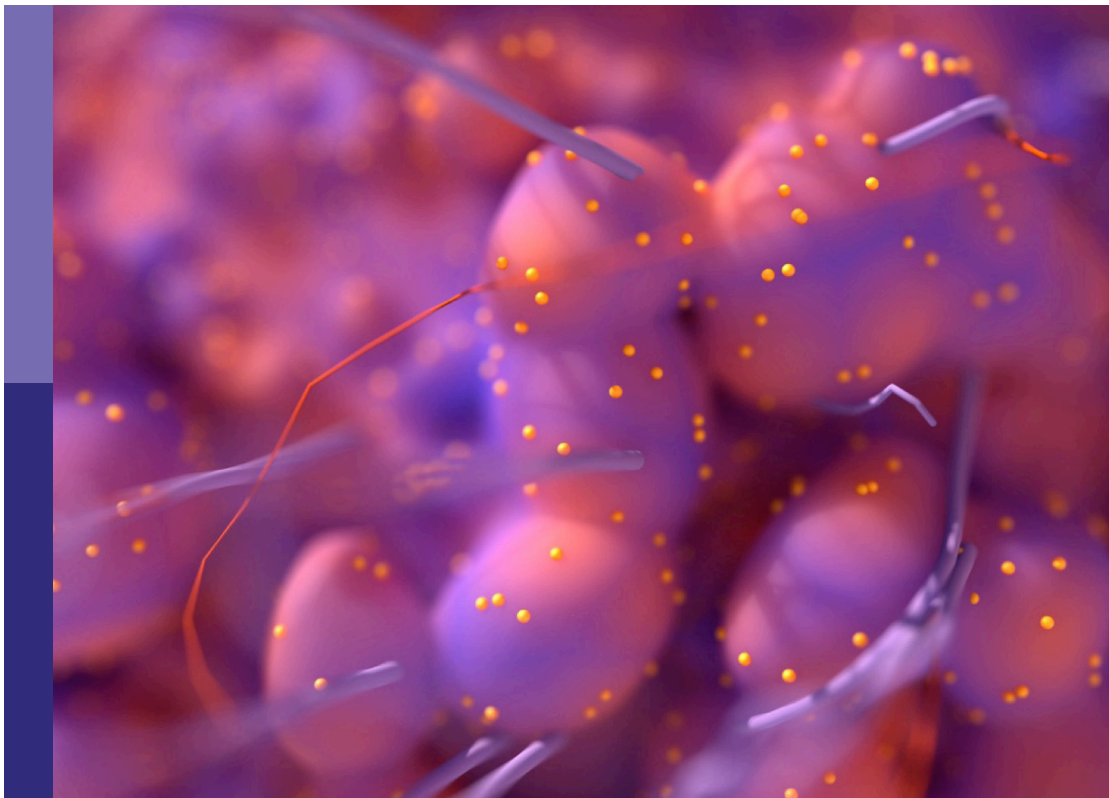
Published in
Frontiers in Oncology



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ISSN 1664-8714
ISBN 978-2-83250-953-1
DOI 10.3389/978-2-83250-953-1

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Cardiotoxicity induced by radiotherapy and/or chemotherapy after cancer treatment.

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Citation

Monceau, V., Azimzadeh, O., Boerma, M., Pasinetti, N., eds. (2022). *Cardiotoxicity induced by radiotherapy and/or chemotherapy after cancer treatment*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-83250-953-1

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frontiers | Frontiers in Oncology

TYPE Editorial
PUBLISHED 22 November 2022
DOI 10.3389/fonc.2022.1087928

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OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Radiation Oncology, a section of the journal Frontiers in Oncology

RECEIVED 02 November 2022
ACCEPTED 04 November 2022
PUBLISHED 22 November 2022

CITATION
Boerma M, Azimzadeh O, Pasinetti N and Monceau V (2022) Editorial: Cardiotoxicity induced by radiotherapy and/or chemotherapy after cancer treatment.
Front. Oncol. 12:1087928.
doi: 10.3389/fonc.2022.1087928

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Editorial: Cardiotoxicity induced by radiotherapy and/or chemotherapy after cancer treatment

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KEYWORDS

chemotherapy, targeted therapy, cardiotoxicity, radiotherapy, cancer treatment

Editorial on the Research Topic

Cardiotoxicity induced by radiotherapy and/or chemotherapy after cancer treatment

Introduction

It is our privilege to present 11 articles inthis Frontiers Oncology Research Topic on "*cardiotoxicity induced by radiotherapy and/or chemotherapy after cancer treatment*". The therapeutic management of cancer has progressed over the last 10 years and has led to a significant increase in patient survival rates. Radiotherapy (RT) is classically applied in multiple fractions administered over several weeks to kill cancer while sparing normal tissue as much as possible. However, the corollary is the development of delayed toxicities that alter the quality of life of patients. Chemo- and/or RT-induced cardiovascular disease (CVD) is recognized as a worrisome side effect in patients with thoracic cancers.

This toxicity appears to be amplified if there are comorbidities as presented in the article by Banfill et al. that shows the effect of radiation dose on cardiac death after thoracic RT is different in patients with and without cardiac comorbidities. Therefore, CVD risk factors should be identified and managed in conjunction with RT for lung cancer. In the same context, Mirestean et al. describe the concerns that should be considered in breast cancer (BC) therapy. BC is the most common cancer in women worldwide, often treated with RT including whole breast irradiation (WBI). Recent data recommend the use of hypofractionation (HF)-WBI regardless of age or stage of disease. However, some studies reported an increased incidence of cardiac death with HF-WBI, particularly in patients with pre-existing cardiac risk factors at the time of treatment. There is also a need to develop

multivariable radiobiologic models including histologic, molecular, and clinical parameters to identify risk groups and dosimetric tolerance to limit the incidence of late cardiac events. Abravan et al. describe the use of real-world data (RWD) to study radiation-induced heart disease (RIHD) in lung cancer patients, summarizing how heart dosimetric factors associate with outcome, strength, and limitations of the RWD studies, and how RWD can be used to assess a change to cardiac dose constraints. Since RT of BC can result in an increased risk of long-term major CVD events, it is critical to detect subclinical left ventricular (LV) dysfunction early in RT treated BC patients to determine the dose-response relationships between cardiac doses and these events. The results of Locquet et al. highlight that all cardiac doses were strongly associated with the development of subclinical LV dysfunction 6 months after RT. It remains to be determined whether global longitudinal strain measurements at baseline and 6 months after RT can predict subclinical events occurring 24 months after RT. In addition, few studies suggested that RT of BC can induce cardiac arrhythmias and conduction disorders. However, the association between mean heart dose and doses to cardiac substructures is less studied. Errahmani et al. performed an exploratory investigation on BC patients treated with RT, which is the first study suggesting that irradiation of the right atria may require special attention regarding the risk of cardiac arrhythmia and conduction disorders. Regardless of the technique used, during RT, healthy tissues located in the irradiation fields are exposed. This is the main reason for the development of RT techniques in the treatment of left-sided BC, where conventional RT leads to cardiac radiation exposure, such as the deep inspiration breath-hold (DIBH), as described by Lu et al. Whether the DIBH regimen is an optimal solution for left-sided BC remains unclear. This meta-analysis aimed to elucidate the differences between DIBH and free-breathing for patients receiving RT for left-sided BC and provide a practical reference for clinical practice. This study suggests more widely use of DIBH in clinical practice because of its excellent dosimetric performance. An article by Kimpe et al. deals with the social impact of long-term effects of radiation-induced cardiotoxicity as an important concern during the treatment of BC. This study applied health economic modelling techniques to estimate attributed CVD-related costs and disutility. Their analyses suggest that the cost of past investments to achieve the mean heart dose (MHD) in current practice seems justified when considering the gains from cost resulting from radiation-induced CVD events. The question remains to be answered is whether costs associated with further investments in technological advancements offset the expected benefit from reducing the MHD. Altogether, epidemiological and clinical data underline the importance of cardiac side effects after RT, but the pathophysiological, cellular and molecular bases of such side effects remain poorly understood. This Research Topic highlights research that creates new knowledge of cellular and molecular signaling pathways using preclinical animal models. To address the impact of genetic bases and disparate responses to RT, Choudens et al. used Dahl salt-sensitive rats as a surrogate hypertension model to analyse the injury to the heart by irradiation. Similarly using a rat model of lung irradiation, Wiedemann et al. demonstrated a series of pathologies following remodeling of the pulmonary vasculature. The similarities between the mechanisms of vascular remodeling in pulmonary hypertension and those after irradiation could be translated into interventions that benefit patients treated for thoracic tumors, where radiation to lung tissue often cannot be avoided. This knowledge greatly facilitates the discovery of biomarkers for cardiovascular toxicity, the identification of new cardioprotective therapeutic targets, and the optimization of prevention and intervention strategies in chemo- and RT. Cancer immune checkpoint inhibitors have led to recent advances in the field of cancer immunotherapy improving overall survival in multiple cancers with historically abysmal prognoses. Cardiac-specific immune-related adverse events are potentially fatal. However, the understanding of autoimmune cardiotoxicity remains limited due to its rareness. Irabor et al. provide a literature review on the pathologic mechanisms, diagnosis, and management of autoimmune cardiotoxicity resulting from ICIs and offer a perspective on potential strategies and research to prevent and mitigate their occurrence. Thoracic RT has been associated with increased cardiac morbidity and mortality in numerous studies, including the landmark Darby´s study (2013), demonstrating a linear increase in cardiac mortality with increasing mean cardiac radiation dose. However, the extent to which cardiotoxicity has been incorporated as an endpoint in RT studies has not been standardized and is sometimes unknown. To better characterize cardiac toxicities, future prospective studies involving thoracic RT should include cardiotoxicity endpoints as recommended in the study by Prasad et al.. We hope that this Research Topic will arouse the interest of radiation researchers, epidemiologists and clinicians to continue to pursue research that increases our knowledge on CVD following chemo- and RT, eventually implementing practices that will improve the safety of cancer therapy.

Authors contributions

All authors listed have contributed equally. VM drafted the manuscript and incorporated the ideas of all authors. MB, OA and NP provided comments and approved of the final version.

Acknowledgments

The authors would like to thank all scientists who contributed to this Research Topic as well as various reviewers/editors of the respective manuscripts, for their efforts, timely responses and

enthusiasm. We also thank the Frontiers Editorial Office for their assistance and support.

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frontiers in Oncology

ORIGINAL RESEARCH
published: 09 February 2022
doi: 10.3389/fonc.2022.808531

Lack of Cardiotoxicity Endpoints in Prospective Trials Involving Chest Radiation Therapy: A Review of Registered, Latter-Phase Studies

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OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Radiation Oncology, a section of the journal Frontiers in Oncology

Received: *03 November 2021*
Accepted: *20 January 2022*
Published: *09 February 2022*

Citation:
Prasad RN, Miller ED, Addison D and Bazan JG (2022) Lack of Cardiotoxicity Endpoints in Prospective Trials Involving Chest Radiation Therapy: A Review of Registered, Latter-Phase Studies. Front. Oncol. 12:808531. doi: 10.3389/fonc.2022.808531

Background: Chest radiation therapy (RT) has been associated with increased cardiac morbidity and mortality in numerous studies including the landmark Darby study published in 2013 demonstrating a linear increase in cardiac mortality with increasing mean heart radiation dose. However, the extent to which cardiotoxicity has been incorporated as an endpoint in prospective RT studies remains unknown.

Methods: We queried clincaltrials.gov to identify phase II/III trials in lung, esophageal, lymphoma, mesothelioma, thymoma, or breast cancer from 1/1/2006-2/1/2021 enrolling greater than 100 patients wherein chest RT was delivered in at least one treatment arm. The primary endpoint was the rate of inclusion of cardiotoxicity as a specific primary or secondary endpoint in the pre- (enrollment started prior to 1/1/2014) versus post-Darby era using the Chi-square test ($p<0.05$ considered significant). We also analyzed clinical trial factors associated with the inclusion of cardiotoxicity as an endpoint using logistic regression analysis.

Results: In total, 1,822 trials were identified, of which 256 merited inclusion. 32% were for esophageal, 31% lung, 28% breast, and 7% lymphoma/thymoma/mesothelioma cancers, respectively. 5% (N=13) included cardiotoxicity as an endpoint: 6 breast cancer, 3 lung cancer, 3 esophageal cancer, and 1 lymphoma study. There was no difference in the inclusion of cardiotoxicity endpoints in the pre-Darby versus post-Darby era (3.9% vs. 5.9%, P=0.46). The greatest absolute increase in inclusion of cardiotoxicity as an endpoint was seen for lung cancer (0% vs. 6%, p=0.17) and breast cancer (5.7% vs. 10.8%, p=0.43) studies, though these increases remained statistically non-significant. We found no clinical trial factors associated with the inclusion of cardiotoxicity as an endpoint.

Conclusions: Among prospective trials involving chest RT, cardiotoxicity remains an uncommon endpoint despite its prevalence as a primary source of toxicity following treatment. In order to better characterize cardiac toxicities, future prospective studies involving chest RT should include cardiotoxicity endpoints.

Keywords: chest radiation therapy, thoracic radiation therapy, cardiotoxicity, clinical trials, major adverse cardiac events, breast cancer, esophageal cancer, lung cancer

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of mortality in cancer survivors (1). Chest radiation therapy (RT) has been associated with increased rates of cardiac morbidity and mortality in survivors of breast (2–4), lung (5, 6), and esophageal cancers (7–9), and lymphoma (10–13) including by the landmark Darby study published in 2013 that demonstrated a linear, persistent increase in cardiac mortality with increasing mean heart radiation dose in patients treated for breast cancer (3). The Darby study was a population-based, case control analysis of 2,168 Scandinavian women who underwent RT for breast cancer from 1958-2001, 963 of whom experienced major coronary events while the remainder without coronary events served as controls (3). This analysis established that excess, major coronary events occurred even within the first 5 years after RT and that these risks persisted for decades.

After publication of this study, increased awareness of this issue motivated numerous other retrospective analyses, including reports in non-small cell lung cancer (NSCLC) demonstrating that a significant proportion of cardiac events occur even within two years of RT completion (5, 6). Additionally, using echocardiography, cohort studies have identified subclinical cardiac dysfunction occurring just months after RT (14, 15). Appreciation of the adverse effects of RT on the heart, largely based on retrospective studies, has led to an increased emphasis on minimizing radiation dose to the heart or its substructures during radiation planning (3, 5, 6, 9, 12, 13, 16). However, the extent to which cardiotoxicity has been incorporated as an endpoint in prospective RT studies remains unknown. We hypothesized that cardiotoxicity has increased in frequency as an endpoint in oncology trials involving thoracic RT since publication of the Darby study in 2013, and we sought to quantify this change in trial design.

METHOD

We queried clinicaltrials.gov for all phase II or III interventional studies conducted from 1/1/2006 until 2/1/2021 that included RT for definitive therapy of breast, esophageal, lymphoma, mesothelioma, thymoma, or lung cancer with a planned enrollment of greater than 100 patients. In our query, we specified interventional studies of phase II or III only within the relevant date range and included "radiation" and the type of cancer as additional terms. Smaller, observational, and early-phase studies were excluded. While single arm, observational studies looking at serum and imaging biomarkers provide important hypothesis-generating data, it is difficult to correlate these studies with cardiac-specific outcomes due to the number of patients needed to see any measurable increase in cardiac toxicity. Absolute rates of excess major cardiac events may be low, particularly in certain populations such as patients with breast cancer (4) or lymphoma, and identifying meaningful differences may be beyond the scope of smaller trials. It often takes years for events to occur, which is beyond the funding window of many smaller, early-phase studies that are just looking at early changes in biomarkers. Focusing on phase II/III trials mitigated these issues, and allowed us to assess the rate at which the trials most likely to affect standard of care were considering the potential cardiac consequences of interventions. In contrast, phase IV trials were not considered, because we sought to quantify the rate of inclusion of cardiotoxicity endpoints in the pre-market trials that impact standard of care in oncology. Studies returned by clinicaltrials.gov query were evaluated by an experienced team of 3 researchers with a background in clinical radiation oncology. Studies were stratified into pre-Darby era (enrollment started prior to 1/1/2014) or post-Darby era (enrollment after 1/1/2014). Additional information about each trial was collected, including but not limited to trial phase, type of primary endpoint (cancer control, patient reported toxicity, physician reported toxicity, or other), presence of a cardiotoxicity primary or secondary endpoint, detailed information about RT fractionation and delivery, inclusion of concurrent systemic therapy, current trial recruitment status, country of origin, trial duration, and trial sponsor. We included the report of any cardiac endpoints by trials including both clinical and subclinical outcomes measures.

The primary endpoint of our analysis was the rate of inclusion of any cardiotoxicity endpoint (whether primary or secondary) in trials from the pre-Darby versus post-Darby eras. The Chi-square test was used to compare the rate of inclusion of cardiotoxicity endpoints between groups. We further analyzed whether any clinical trial factors (disease site – breast vs. non-breast; study era – post-Darby vs. pre-Darby; clinical trial phase – III vs. I-II, sample size – dichotomized by the median sample size across all trials; trial duration – dichotomized by the median; and use of concurrent chemotherapy – yes vs. no) were associated with the inclusion of cardiotoxicity as an endpoint with univariate logistic regression analysis. Statistical tests were 2-sided with statistical significance evaluated at the α=0.05 significance level.

RESULTS

Overall, 1,822 trials were reviewed, and 256 met the study criteria (**Figure 1**). Of the trials included, 32%, 31%, and 28% involved

esophageal, lung, and breast cancers, respectively; detailed characteristics of the included trials, stratified by trial era, are presented in **Table 1**. The remaining 9% were lymphoma, thymoma, or mesothelioma. Across all trials, 59% included concurrent systemic therapy, while 4% and 4% of trials included stereotactic body radiation therapy and proton therapy, respectively. Overall, 5% of included trials (N=13) included cardiotoxicity as an endpoint: 6 breast cancer (8%), 3 lung cancer (4%), 3 esophageal cancer (4%), and 1 lymphoma study (4% of all other included cancers) (**Figure 2A**). The median trial duration was 6.5 years (1.0 - 19.9 years). Of these trials, 5 (2%) included a cardiotoxicity metric as a primary endpoint and 8 (3%) as a secondary endpoint. In general, these endpoints were clinically defined and predominately involved the measurement of serious late effects such as major adverse cardiac events (7 trials, or 54%), including cardiac death and/or ischemic heart disease. A minority of trials evaluated for lower-grade cardiac toxicities. Across all cancer types, there was no statistically significant increase in the inclusion of cardiotoxicity as an endpoint in the pre-Darby versus post-Darby era (3.9% vs. 5.9%, p=0.46). The greatest absolute increases in inclusion of cardiotoxicity as an endpoint were seen for lung cancer (0.0% vs. 5.9%, p=0.17) and breast cancer (5.7% vs. 10.8%, p=0.43) studies, though these increases remained insignificant (**Figure 2B**). Inclusion of cardiotoxicity endpoints in studies of esophageal cancer decreased from 3.8 to 3.6%, and from 7.7% to 0.0% for all other included malignancies. On univariate logistic regression analysis, no variables of interest were associated with increased likelihood of a trial reporting a cardiotoxicity endpoint (**Table 2**). Multivariate analysis was not pursued due to the lack of significant variables on univariate analysis.

DISCUSSION

Recent recognition of the adverse cardiac effects of RT has led to an increased focus on minimizing radiation dose to the heart or its substructures (3, 5, 6, 9, 12, 13, 16), yet overall, our analysis found no significant increase in the rate of cardiotoxicity endpoints in clinical trials involving chest RT in the post-Darby era. Even after publication of the Darby study in 2013, roughly 95% of included trials did not include cardiotoxicity endpoints. Examined individually, none of the included malignancies showed a significant change in cardiac endpoint reporting over time. There was a numeric increase in the proportion of trials in breast and lung cancer including cardiotoxicity endpoints, perhaps related to increased awareness from retrospective series and subsequent improved access to funding, but it was ultimately non-significant and overall rates remained objectively low. Surprisingly, studies in the lymphoma/thymoma/mesothelioma showed a numeric decrease in incorporation of cardiotoxicity endpoints over time which most likely relates to the small sample size. These results are startling given the recent trend towards increasing publication of retrospective data detailing the prevalence of cardiac toxicity after chest RT. Of the 250 Pubmed indexed publications identified by a query for "radiation therapy" AND "cardiotoxicity," over half (128) were published in the last 5 years alone. One possible explanation is that investigators have been prematurely reassured by decreased total doses to the heart seen with modern treatment planning and delivery techniques in breast (17), esophageal (18), and lung (19) cancers. However, even if heart doses are decreasing, it is still important that we adequately monitor cardiac outcomes, so that we can confirm that these lower doses translate to decreased cardiac risk. Ultimately, if cardiac events are adequately measured in the prospective setting and event rates are found to be acceptably low with modern RT techniques, attempts at further decreasing cardiac dose through expensive therapies like proton and heavy ion therapy may be unnecessary. Retrospective studies are useful for hypothesis generation, but due to the inherent biases of such studies, greater prospective characterization of cardiotoxicity after RT is needed.

These findings are highly concerning, because CVD remains the leading cause of non-cancer mortality in cancer survivors (1), and chest RT is consistently linked to increased cardiac complications in survivors of numerous cancers (2–13) perhaps occurring as early as within 2 years of RT completion (5, 6) with elevated risk persisting for decades (2–13). Studies following survivors of various thoracic and chest malignancies suggest that the risk of cardiac complications increases linearly with increasing heart radiation dose (3, 13). Possible complications vary widely depending upon the damaged substructure but include pericarditis (pericardium), heart failure (myocardium), acute coronary syndrome (coronary arteries), valvular disease, and arrhythmia (conduction system) (16).

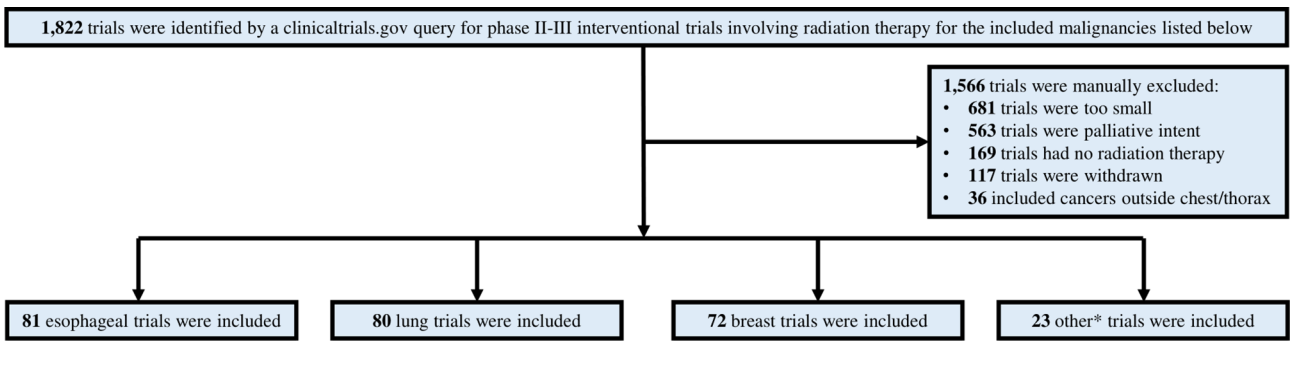

FIGURE 1 | Study schema documenting identification of trials meeting inclusion criteria. *other = lymphoma, mesothelioma, and thymoma.

TABLE 1 | Characteristics of trials meeting inclusion criteria, stratified by era (pre- vs post-Darby publication).

	All Trials N (%)	2013 or Earlier N (%)	2014 or Later N (%)
Total	256 (100)	104 (41)	152 (59)
Cancer Type			
Esophagus	81 (32)	26 (25)	55 (36)
Lung	80 (31)	30 (29)	50 (33)
Breast	72 (28)	35 (34)	37 (24)
Other	23 (9)	13 (13)	10 (7)
Trial Phase			
II	122 (48)	54 (52)	68 (45)
III	134 (52)	50 (48)	84 (55)
Primary Endpoint			
Cancer Control	199 (78)	78 (75)	121 (80)
Patient Reported Toxicity	7 (3)	3 (3)	4 (3)
Physician Reported Toxicity	41 (16)	19 (18)	22 (14)
Other	8 (3)	4 (4)	5 (3)
Cardiotoxicity Endpoint			
Primary	5 (2)	1 (1)	4 (3)
Secondary	8 (3)	3 (3)	5 (3)
None	243 (95)	100 (96)	143 (94)
Concurrent Systemic Therapy			
Yes	152 (59)	53 (51)	99 (65)
No	104 (41)	51 (49)	53 (35)
Planned Enrollment			
100-499	202 (79)	81 (78)	121 (80)
500-999	35 (14)	13 (13)	22 (14)
>1000	18 (7)	9 (9)	9 (6)
SBRT[a] Included			
Yes	9 (4)	2 (2)	7 (5)
No	247 (96)	102 (98)	145 (95)
Proton Therapy Included			
Yes	10 (4)	7 (7)	3 (2)
No	246 (96)	97 (93)	149 (98)

[a]*Stereotactic body radiation therapy.*

These risks are in addition to known cardiac risks from chemotherapy, and in the modern era, additive risk from concurrent or sequential systemic novel immunotherapies and targeted therapies must also be considered given their increasing links to development of cardiovascular disease (20). The majority of studies included in this analysis included concurrent chemotherapy, immunotherapy, targeted therapy, or hormone therapy.

This analysis has several limitations. This study only evaluated the endpoints from definitive phase II or III trials involving thoracic RT in patients treated with definitive intent with at least 100 patients. The reason for this is that although the Darby et al. study demonstrated a relative increase in at least one acute coronary event of 7.4% per Gray mean heart dose, the absolute increases are quite small especially in patients with no cardiac risk factors. For instance, the Darby et al. study estimates that for a healthy 40-year old woman that receives a mean heart dose of 2 Gy during her breast RT, her risk of at least one acute coronary event by the time she is 80 years old increases by an absolute value of 0.7% (3). Similarly, a follow-up study by Taylor et al. demonstrates that the absolute increase in the risk of death from ischemic heart disease for a healthy 50-year old woman that receives as much as 4 Gy mean heart dose is only 0.3% by the time she is 80-years old (4). This underscores the point that large numbers of patients need to be followed for long periods of time in order to adequately capture the potential effects of thoracic RT on cardiac toxicities. As a result, we did not include single arm prospective studies aimed at identifying serum and/or imaging biomarkers of thoracic RT, because these studies involve small numbers of patients and have limited follow-up to correlate these biomarkers with actual cardiac toxicity. Therefore, not only is it likely that the types of clinical trials that we did not include in our analysis would not have significant rates of inclusion of cardiotoxicity endpoints, but it is also likely that these types of smaller interventional trials would not have adequate power to identify small increases in cardiac toxicities above expected baseline rates. No additional study registries were queried for trials involving chest RT, but in order to best reflect widespread practice, we focused on clinical trials evaluating definitive oncologic therapy as captured by the US-based clinicaltrials.gov. Additionally, study protocols could have been reviewed to determine the rate at which trials without cardiac endpoints were still monitoring for adverse cardiac events. However, such an approach has limited utility, as prior work suggests that cardiotoxicity is underreported by clinical trials that are not specifically designed to characterize cardiac events (21–23). Dichotomizing the comparison eras differently may have impacted the significance of the trend in inclusion of endpoints over time but would not affect the overarching conclusion that clinical trials including cardiac outcomes are too rare.

In summary, among prospective clinical trials involving chest RT, cardiotoxicity remains an uncommon endpoint despite its prevalence as a primary source of toxicity following treatment.

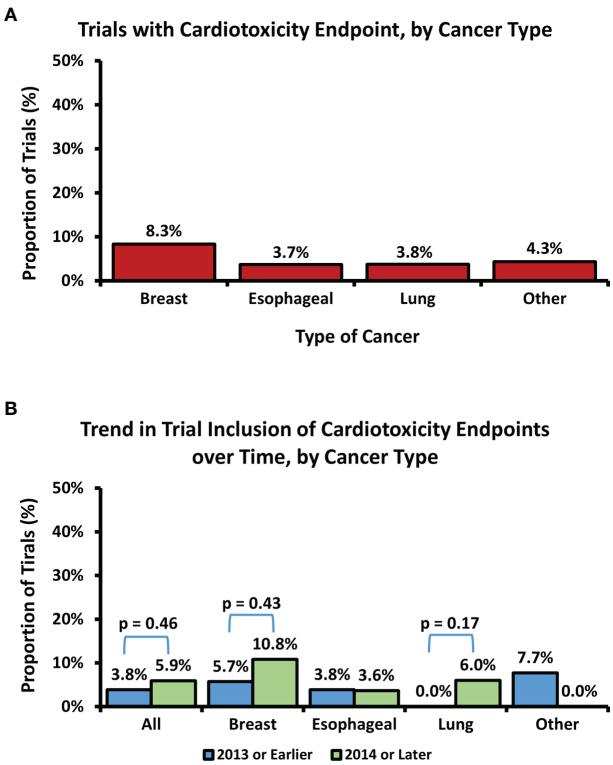

FIGURE 2 | Overall proportion of included trials with a cardiotoxicity endpoint, by cancer type **(A)**. Trend in trial inclusion of cardiotoxicity endpoints over time, by cancer type **(B)**. Differences in rate of inclusion of cardiac endpoints over time are non-significant ($p > 0.05$).

TABLE 2 | Logistic regression analysis of association of radiation clinical trial characteristics and inclusion of a cardiac primary or secondary endpoint.

Variable	Odds Ratio	95% CI	p-value
Breast vs. Non-Breast disease site	2.30	0.75-5.25	0.15
Phase III vs. Phase II	2.12	0.64-7.08	0.22
Use of Concurrent Chemotherapy (Yes vs. No)	0.41	0.13-1.29	0.13
Trial Size (>220 patients vs. ≤ 220 patients)	1.20	0.39-3.66	0.75
Trial Duration (>6.5 years vs. ≤ 6.5 years)	0.43	0.13-1.42	0.17
Post-Darby Era vs. Pre-Darby Era	1.57	0.47-5.25	0.46

While inclusion of cardiotoxicity endpoints has increased slightly over time (numeric increase not achieving significance), overall rates of inclusion among latter-phase trials remain objectively low. Thus, in order to better characterize cardiac toxicities, education is needed to increase researchers' and clinicians' awareness of this subject. Additionally, future prospective studies involving chest RT should include cardiotoxicity endpoints with greater frequency.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

RP and EM: first authors. DA: contributor. JB: senior authorship. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported in part by National Cancer Institute grant P30 CA016058. Dr. Addison is supported by the following grants from the National Institutes of Health: K12CA133250 and K23-HL155890. Dr. Addison is also supported by an American Heart Association–Robert Wood Johnson Foundation Faculty Development Program grant.

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frontiers in Oncology

ORIGINAL RESEARCH
published: 02 March 2022
doi: 10.3389/fonc.2022.828177

Lisinopril Mitigates Radiation-Induced Mitochondrial Defects in Rat Heart and Blood Cells

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OPEN ACCESS

Edited by:
Virginie Monceau, Institut de Radioprotection et de Sûreté Nucléaire, France

Reviewed by:
Vasily Yakovlev, Virginia Commonwealth University, United States
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Specialty section:
This article was submitted to Radiation Oncology, a section of the journal Frontiers in Oncology

Received: *03 December 2021*
Accepted: *31 January 2022*
Published: *02 March 2022*

Citation:
Ortiz de Choudens S, Sparapani R, Narayanan J, Lohr N, Gao F, Fish BL, Zielonka M, Gasperetti T, Veley D, Beyer A, Olson J, Jacobs ER and Medhora M (2022) Lisinopril Mitigates Radiation-Induced Mitochondrial Defects in Rat Heart and Blood Cells. Front. Oncol. 12:828177. doi: 10.3389/fonc.2022.828177

The genetic bases and disparate responses to radiotherapy are poorly understood, especially for cardiotoxicity resulting from treatment of thoracic tumors. Preclinical animal models such as the Dahl salt-sensitive (SS) rat can serve as a surrogate model for salt-sensitive low renin hypertension, common to African Americans, where aldosterone contributes to hypertension-related alterations of peripheral vascular and renal vascular function. Brown Norway (BN) rats, in comparison, are a normotensive control group, while consomic SSBN6 with substitution of rat chromosome 6 (homologous to human chromosome 14) on an SS background manifests cardioprotection and mitochondrial preservation to SS rats after injury. In this study, 2 groups from each of the 3 rat strains had their hearts irradiated (8 Gy X 5 fractions). One irradiated group was treated with the ACE-inhibitor lisinopril, and a separate group in each strain served as nonirradiated controls. Radiation reduced cardiac end diastolic volume by 9-11% and increased thickness of the interventricular septum (11-16%) and left ventricular posterior wall (14-15%) in all 3 strains (5-10 rats/group) after 120 days. Lisinopril mitigated the increase in posterior wall thickness. Mitochondrial function was measured by the Seahorse Cell Mitochondrial Stress test in peripheral blood mononuclear cells (PBMC) at 90 days. Radiation did not alter mitochondrial respiration in PBMC from BN or SSBN6. However, maximal mitochondrial respiration and spare capacity were reduced by radiation in PBMC from SS rats (p=0.016 and 0.002 respectively, 9-10 rats/group) and this effect was mitigated by lisinopril (p=0.04 and 0.023 respectively, 9-10 rats/group). Taken together, these results indicate injury to the heart by radiation in all 3 strains of rats, although the SS rats had greater susceptibility for mitochondrial dysfunction. Lisinopril mitigated injury independent of genetic background.

Keywords: cardiotoxicity, thoracic radiation, mitochondrial dysfunction, rat model, lisinopril

INTRODUCTION

Breast and lung cancer accounts for 28% of new cancer diagnoses in the United States, and 29% of cancer deaths (1). Radiation therapy is an essential part of treatment for these malignancies, with more than 50% of patients receiving radiation (2). Exposure of radiation to thoracic structures can cause a wide variety of acute symptoms and delayed toxicities, including cardiac injuries (3). Radiation to the heart is often unavoidable when treating lung, breast, esophageal, and other thoracic malignancies. Efforts to increase treatment doses are limited by normal tissue tolerance. With the increasing role of radiation therapy in the contemporary treatment of cancer, patients that are long term survivors are at risk of cardiovascular injury and mortality (2, 3). High-dose radiation exposure to the heart can cause cardiac dysfunction, developing months to decades following treatment (4, 5). This includes injury to the cardiac tissues and vasculature, which can lead to complications such as pericarditis, coronary artery disease, ischemic heart disease, congestive heart failure, conduction defects and valvular dysfunction (6, 7).

One of the features of cardiac injury is the formation of fibrosis, distinguished by collagen deposition both inside and surrounding cardiomyocytes (4, 7–9). Radiation induced cardiotoxicity can range from issues with contractility, nerve impulse transmission, fibrosis, and compliance, resulting in arrhythmia and heart failure. Additional causes of cardiac injury include endothelial cell damage and activation of inflammatory and atherosclerotic responses (4, 7, 8, 10–15). When examining heart and lung irradiation in rats, Ghobadi et al. (16) concluded that combined irradiation of lung and heart induced pronounced increases in left ventricular end-diastolic pressure and relaxation time, in addition to an increase in right ventricle end-diastolic pressure, indicative of biventricular diastolic dysfunction (16).

Advances in imaging and radiotherapy delivery techniques have helped to reduce cardiac exposure (17–22). However, there is no known safe dose for cardiac exposure, and heart radiation exposure often remains unavoidable (4). The underlying causes and biomarkers of radiation-induced cardiotoxicity are currently unknown, prompting the need for experimental models with inherent differences in sensitivity and resistance to the development of radiation-induced cardiotoxicity (19). There have been numerous preclinical cell and animal models that have been used to study the mechanisms behind radiation-induced heart dysfunction (2, 4). Nonetheless, the mechanism of cardiac injury has not yet been fully elucidated (4). By improving our understanding of the biological pathways and mechanisms involved in radiation induced normal tissue toxicity, cancer treatment can be improved, with the goal of achieving maximum therapeutic benefit and reduced toxicities (2).

Preclinical rat models have been used to decipher genetic and molecular regulation of radiation-induced injury to normal tissues. For example, Dahl salt-sensitive rats (SS rats) develop hypertension and related cardiovascular and kidney diseases when fed a high salt diet (23, 24). The SS rats also exhibit early onset renal dysfunction (25, 26), with low renin activity, as compared to non-salt sensitive strains such as Brown Norway rats (BN rats). Both these strains have been used previously to identify genetic modifiers of radiation induced cardiotoxicity (19). A consomic strain SSBN3, with substitution of rat chromosome 3 from the BN rat strain into the SS background has also been used. Radiation induced cardiotoxicity was more severe in SS rats as compared to BN or SSBN3 rats without altering levels of dietary salt intake. In the current study, we use SS and BN rats along with SSBN6 rats that have a substitution of rat chromosome 6 from the resistant BN strain into the SS background to further explore genetic diversity in radiation sensitivity (27). Such chromosome substitution strains (consomics) have been commonly used to identify genetic loci that modulate response (19). Chromosome 6 from BN to the SS background conferred mitochondrial preservation and cardioprotection during *ex vivo* myocardial ischemia reperfusion (27). Because 73% of hypertensive and 36% of normotensive African Americans have salt sensitivity and low renin activity, compared to 56% of White hypertensive individuals (24–26, 28), SS-rats have been used as surrogates to study hypertension and kidney diseases common to African Americans (24–26). Evidence suggests that the proinflammatory effects of aldosterone contribute to both hypertension and to hypertension-related vascular disease (29). Sensitivity to radiation has not been well studied in diverse populations including African Americans, underscoring the usefulness of the SS rats as models.

The heart is made up of cells that are enriched in mitochondria, which are the powerhouse for myocytes. Mitochondrial derived ATP *via* oxs-phos is a hallmark of physiological cardiac function. With beginning of disease, cardiac metabolism changes to glycolysis in part due to mtDNA damage (30). Under pathological conditions, mitochondria are also a major source of reactive oxygen species, which has been reviewed by Stowe and Camara (31), including exposure to ionizing radiation (32). Radiation injury is mediated by DNA damage, so that extranuclear mitochondrial DNA is an important target. Mitochondria account for up to 30% or more of the cell volume in the heart and certain blood cells (32). Immediately after radiation, transient oxidative stress is generated by radiolysis of water (32, 33), but following that, oxidative stress from intracellular activities such as mitochondrial electron transport systems add to the delayed effects of radiation. Mutations in mitochondrial DNA, as well as changes in intracellular cytokine and signaling cascades induced by radiation, generate waves of oxidative stress that lead to cell death and apoptosis. This pathological process can continue for months post-irradiation (32). Partial deactivation of mitochondrial respiratory complexes have been reported in irradiated mouse

Abbreviations: ACEi, Angiotensin Converting Enzyme Inhibitor; ANOVA, Analysis of Variance; AST, Antero Septal Thickness; ATP, Adenosine Tri Phosphate; BHI, Bioenergetic Health Index; BN, Brown Norway; DNA, Deoxy Ribonucleic Acid; DPBS, Dulbecco's Phosphate Buffered Saline; EDTA, Ethylene Diamine Tetra Acetic acid; EDV, End Diastolic Volume; EF, Ejection Fraction; ESV, End Systolic Volume; FCCP, tri Fluoromethoxy Carbonyl Cyanide Phenylhydrazone; Gy, Unit of Radiation dose; ILT, Infero Lateral Thickness; IVSD, Inter Ventricular Septum Diastole; LV, Left Ventricle; LVIDd, Left Venticular Internal Dimension Diastole; LVPWD, Left Ventricle Posterior Wall Diastole; OCR, Oxygen Consumption Rate; PBMC, Peripheral Blood Mononuclear Cells; RIHD, Radiation Induced Heart Disease; RPMI, 1640 Cell culture medium; SS, Dahl salt-sensitive; SSBN6, Consomic rat with substitution of rat chromosome 6 from BN rat on SS background; SV, Stroke volume.

hearts weeks after irradiation (34). More recently, cellular oxidative stress has been measured by the 'Bioenergetic Health Index (BHI)' (35). Using high-throughput assays to measure oxygen consumption, cellular bioenergetics are described to serve as a sensitive biomarker of health. Changes such as mitochondrial dysfunction have been reported in diabetes, cardiovascular disease, cancer, and toxic chemical exposures (36). Interestingly, mitochondrial activity in cells throughout the body was found to be altered by diseases associated with a specific organ, including diabetes and neurodegeneration (36–39). Since circulating peripheral blood mononuclear cells (PBMC) are easily accessible, the BHI in PBMC could potentially serve as a biomarker of organ diseases, such as cardiotoxicity, after localized radiation to the heart and lung.

Angiotensin-converting enzyme inhibitors have been found to mitigate many of the delayed injuries to cardiac and pulmonary systems (16, 40), and decrease functional and structural damage in irradiated hearts (41). In humans, ACE inhibitors are known to protect the heart from remodeling by reducing the effects of angiotensin II (42–45). We hypothesize that radiation to the heart will cause cardiac injury that will be evidenced by functional changes (monitored by echocardiogram) in our rat models. This injury will be reduced by the addition of ACE inhibitors. Using the changes in echocardiogram parameters we aimed to identify functional changes that occurred in the hearts of irradiated SS, BN and SSBN6 rats, as well as to determine if the changes in mitochondrial bioenergetics in PBMC could serve as a relatively non-invasive biomarker to predict genetically regulated, disparate responses to radiation.

MATERIALS AND METHODS

Animal Models

Small animal models, including rodents, have been used for many decades to study cardiac radiation toxicity, given the physiological similarities that these models have to humans. In order to mirror the radiation doses and fractionations delivered to humans as part of their cancer treatment, we used fractionated radiation including the left lung and heart. To target these organs accurately, image guided radiation was used. The X-Rad SmART research platform (Precision X-ray) is a CBCT image guided system, that allows for irradiation of small experimental animals, and can provide an accuracy that is similar to clinical RT (2, 46).

Three animal models were used for this project. All rats were generated, bred and genotyped in the Medical College of Wisconsin's Genomic Sciences & Precision Medicine Center. Brown Norway (BN) rats were used as the control group to model a radiation resistant strain of rats. Salt Sensitive (SS) rats represent a low renin model that is more likely to be hypertensive. The third group are the SSBN6, which are SS rats with substitution of rat chromosome 6 from the resistant BN rat strain into the SS background, with the goal of attenuating radiation induced cardiotoxicity (27). Using chromosome substitution strains (consomics) has been a strategy used to identify complex genetic modifiers of cardiovascular phenotypes (19). All rats were female because of greater background information regarding response to heart and thoracic irradiation than in males (40, 47). For the BN rat model, 10 rats were assigned to the control group, 10 rats received radiation, 5 rats received lisinopril and 5 rats received both radiation and lisinopril. For the SS rat model, 10 rats were assigned to the control group, 10 rats received radiation, 10 rats received lisinopril and 10 rats received both radiation and lisinopril. For the SSBN6 rats, 5 rats were assigned to the control group, 5 rats received radiation, 3 rats received lisinopril and 5 rats received both radiation and lisinopril.

Animal Care

All procedures in this study were performed according to the American Guidelines for the Ethical Care of Animals and approved by the Institutional Animal Care and Use Committee of the Medical College of Wisconsin.

Animals and Irradiation

SS, BN, and SSBN consomic SSBN6 rats (Medical College of Wisconsin), aged 11 to 13 wk, were randomly allocated to different treatment groups. Left thoracic irradiation was performed using the high-precision image-guided SmART irradiator (Precision X-Ray, North Branford, CT). Rats were anesthetized by 3% isoflurane/room temperature air inhalation for the duration of each treatment. Pilot V1.8 Imaging Software (University Health Network, Toronto, Canada) was used to create two-dimensional projections over 360° to provide computed tomography scans in transverse, sagittal, and frontal views (**Figure 1**). Rats were positioned in the prone position. Radiation was delivered with a 1.5cm diameter circular collimator that encompassed the left lung and the whole heart. The central axis of the beam (isocenter) was set in the center of the heart, with radiation dose to isocenter of 8 Gy × 5 fractions given once daily, with equally weighted parallel opposed beams. Control rats received anesthesia and sham irradiation. Monte-Carlo-based treatment planning was used to precisely calculate irradiation doses (MAASTRO Radiotherapy Clinic). All rats were maintained in single ventilated cages under pathogen-free conditions at the Biomedical Research Center maintained at a temperature of 23°C on a 12-h:12-h light-dark cycle with access to standard diet (0.4% salt) and water (reverse osmosis hyperchlorinated water).

Lisinopril

Rats were given the angiotensin converting enzyme inhibitor (ACEi) lisinopril (40 mg/L in the drinking water for an approximate dose of 24 mg m^{-2} day^{-1}). Lisinopril was started 7 days after radiation and continued until the experiment was terminated. Seven days after irradiation represents the lower limits of time required for identification and screening of individuals and distribution of countermeasure therapy after a bioterrorism event. It is therefore a treatment window with which we have experience for other radiation associated injuries (48, 49).

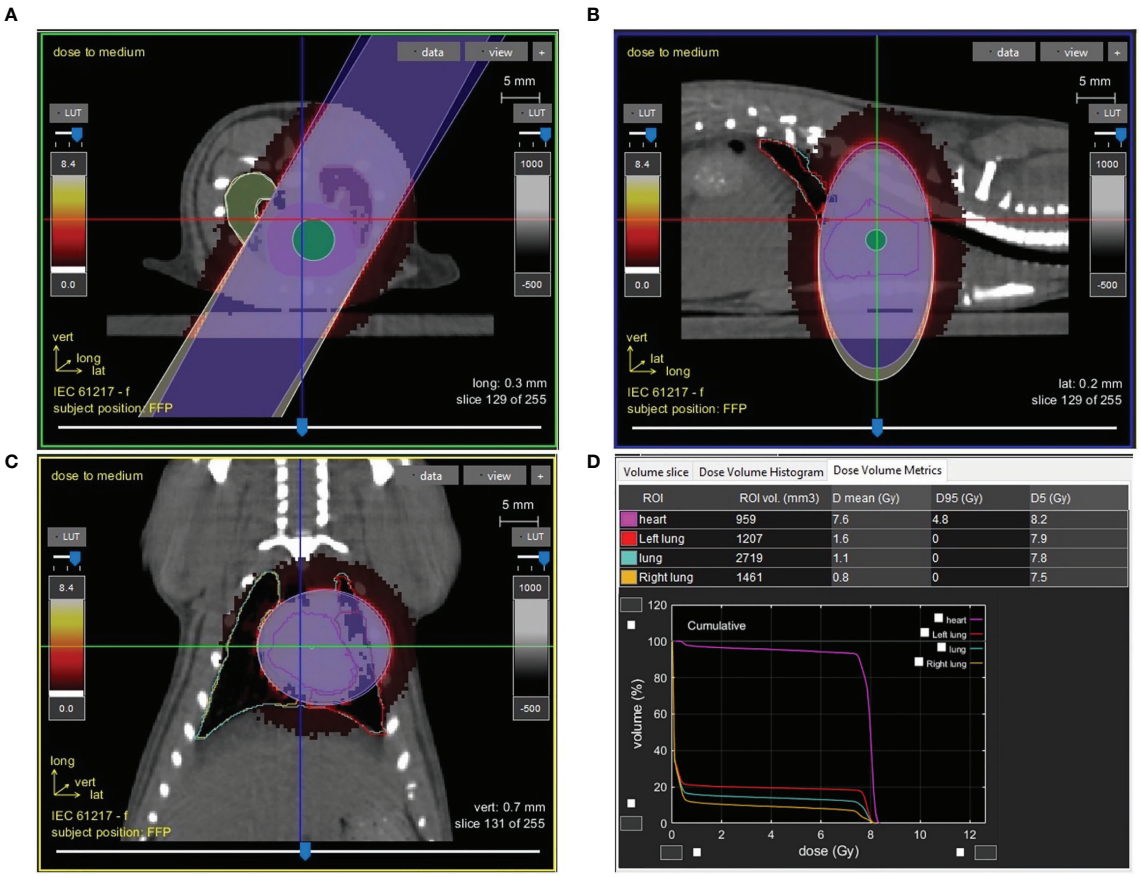


FIGURE 1 | Image guided cardiac irradiation computed tomography images of a representative female rat with a 1.5cm diameter circular collimator plan with radiation dose to isocenter of 8 Gy × 5 fractions with equally weighted parallel opposed beams are shown in the axial **(A)**, sagittal **(B)**, and coronal **(C)** planes. Panel **(D)** shows the dose volume histogram and metrics demonstrating dose to the heart, left lung and right lung.

Echocardiography

Transthoracic echocardiography was performed in anesthetized (2% isoflurane) animals at 60, 90 and 120 days after radiation, or at the corresponding time in nonirradiated, age-matched controls. Measurements and data analyses were performed by an investigator blinded to the study groups. Animals were studied in the left lateral decubitus position with a commercially available echocardiographic system (Vivid 7, General Electric, with an 11-MHz M12-L linear array transducer, GE Healthcare, Waukesha, WI). Transthoracic echocardiography was performed from the cardiac short axis of the left ventricle at the papillary muscle level, using the anatomical M-mode feature of the Vivid 7 echo. An M-mode display was generated from raw data 2D images with the line selected passing through the anterior and inferior segments. Stroke volume (SV) was measured using left ventricular end diastolic volume (EDV) and end systolic volume (ESV) using the formula SV = EDV – ESV. Ejection fraction (EF) was measured using the formula EF = SV/EDV × 100 (50). Cardiac output was calculated by multiplying the heart rate × SV. The LV mass was derived from the anteroseptal thickness (AST) and inferolateral thickness (ILT) using the formula: 0.8 (1.04[(ILT + LVIDd + AST)3 – LVIDd3]) + 0.6 (50). Three consecutive heart beats were measured, and the average used for analysis.

Determination of Blood Bioenergetics in PBMC (Peripheral Blood Mononuclear Cells)

Mitochondrial bioenergetic health can be assessed in circulating platelets and leucocytes, and these values have the potential to be a biomarker for assessing the energetic state of an individual's vital organs. The Agilent Seahorse XF Cell Mito Stress Test is a standard method for assessing mitochondrial bioenergetic function. Evaluations of multiple metrics of mitochondrial function are derived from oxygen consumption rates measured in the presence of a panel of inhibitors, to extrapolate values for non-mitochondrial respiration, basal respiration, maximal respiration, proton leak, ATP production and spare respiratory capacity. Oxygen consumption rates (OCR) were evaluated in PBMC at ~ 90 days after irradiation. Whole blood was serially harvested from the jugular vein of rats, using EDTA as an anticoagulant. PBMC were isolated by gradient centrifugation. Briefly, 1 ml of whole blood was diluted with 2 ml of Dulbecco's Phosphate Buffered Saline (Gibco Cat# 14190-144) and layered

onto 3 ml of Histopaque (Sigma-Cat# 10831). These tubes were centrifuged for 30 min at room temperature at 400×g. After centrifugation, the plasma layer was removed carefully by aspiration under gentle vacuum. The buffy coat containing the white blood cells was transferred to a 15 ml conical tube with a transfer pipette. The cells were washed with 10 ml of DPBS and centrifuged at 500×g for 10 min at room temperature. The cell pellet was resuspended in 1 ml of RPMI-1640 medium (Life Technologies Cat# 31800-022) supplemented with sodium pyruvate (1 mM). An aliquot was further diluted in RPMI-1640 (1:50 v/v) to determine the cell count. The original cell suspension was then adjusted to 3.25×106 cells per ml with RPMI-1640. Equal numbers of cells were transferred to a 96 well Seahorse XF plate (0.1 ml/well), centrifuged (250×g, 5 min) and additional medium aliquot (80 µl/well) was added. The oxygen consumption rate was measured using a Seahorse XF96 Extracellular Flux Analyzer (Agilent, USA) equipped with the Wave Desktop and Controller 2.6.1 version Software (47, 48). OCR was measured in at least triplicate wells at basal condition and after sequential additions of (i) Oligomycin (complex V-ATP synthase inhibitor, 1 µg/ml), (ii) Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP, mitochondrial uncoupler, 1 µM), and (iii) antimycin A (complex III inhibitor, 1 µM) + rotenone (complex I inhibitor, 1 µM). The oligomycin-inhabitable OCR is a measure of the contribution of ATP synthesis to the total OCR. FCCP-induced OCR leads to maximal respiration and enables the determination of the maximum and spare respiratory capacity parameters. Antimycin A + rotenone were added to completely block mitochondrial respiration and determine the contribution of mitochondrial and non-mitochondrial oxygen consuming enzymes to the total OCR values. In addition, the difference between the OCR values after oligomycin and after antimycin A and rotenone injection is a measure of mitochondrial proton leak. The average value for each condition was calculated. After completion of the assays, the medium was aspirated and 20 µl of cell lysis buffer (0.1% Triton X-100, 10 mM Tris-HCl, pH 7.0) was added. The protein content in each well was determined with Bradford reagent (Bio-Rad Cat# 5000002) by measurement of absorbance at 595 nm. The OCR values were expressed as pmoles O_2/min/µg of protein.

Statistics

Repeated measures mixed model with the Kenward-Roger approximation (51) was used for restricted maximum likelihood. The model was run separately for each of the following outcomes: EDV in µL/(kg bpm), IVSD in mm, and LVPWD in cm/kg. The factorial treatments of radiation and Lisinopril were estimated adjusting for day (30:intercept, 60, 90 or 120) and strain (BN:intercept, SS and SSBN6) while two-way interactions were investigated. Each experiment included 230 observations. Analyses were run in SAS 9.4, Analytical Products 15.1.

Differences between groups of bioenergetic respiratory parameters in each strain of rats were tested by Analysis of Variance (ANOVA) followed by All Pairwise Multiple Comparison Procedures (Student-Newman Keul's Method). Student's t-tests were used in some cases to determine differences between 2 parameters. P values <0.05 were determined to be significant.

RESULTS

End Diastolic Volume

Because of differences in rat sizes between strains, EDV values were normalized for weight and heart rate. As outlined in **Figure 2**, for the BN control group at 120 days, the average EDV was 8.73 mL/kg(bpm), compared to 9.97 mL/kg(bpm) in SS control and 9.21 mL/kg(bpm) for SSBN6. Radiation reduced end diastolic volume in all rats (-0.94 mL/kg(bpm), p=0.0013), which represents a 9-11% reduction. Lisinopril did not significantly affect the EDV for any of our strains. **Figure 2** demonstrates the changes in EDV values at 30, 60, 90 and 120 days after radiation. Despite the changes in EDV, the EF remained stable across all groups. **Figure 3** shows EFs over time and as a function of treatment group. SS rats had modestly lower EF than other strains, and taking ACE_i increases the EF in this strain only. There are, however, no time trends in any group.

Interventricular Septal Wall Thickness at End Diastole and Posterior Wall Thickness

We examined reduction in chamber size and/or compliance. IVSD values were normalized to body weight. As outlined in

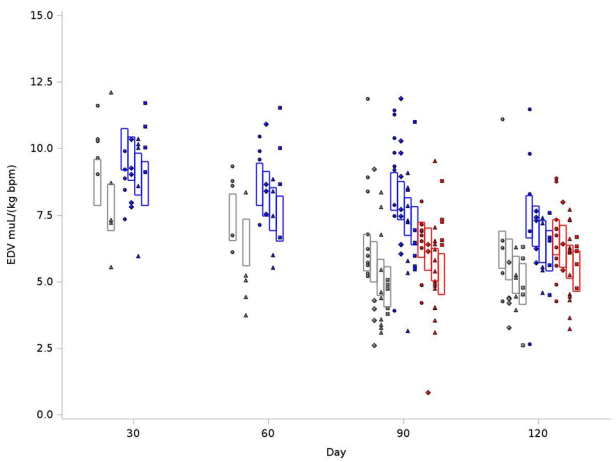


FIGURE 2 | Graphical representation of values for End Diastolic Volumes (EDV) in rats at 4 time points (X-axis). The rats were measured at 4 time points: 30, 60, 90 and 120 days. The values observed are represented by symbols denoting the experimental treatment: Circle (No Radiation and No Lisinopril -R-L), Diamond (No Radiation and Lisinopril -R+L), Triangle (Radiation and No Lisinopril +R-L), or Square (Radiation and Lisinopril +R +L). The colors represent the strain: Brown-Norway (BN: black), Salt-Sensitive (SS: blue) and SS with BN chromosome 6 (SSBN6: red). The rectangles are 95% Confidence Intervals for EDV from a Repeated Measures linear model for treatment that included time, strain, and the SS by day 90 interaction. EDV were normalized to body weight and heart rate and was reduced by 9-11% in all irradiated rats at 120 days (p=0.0013) as compared to their non-irradiated counterparts.

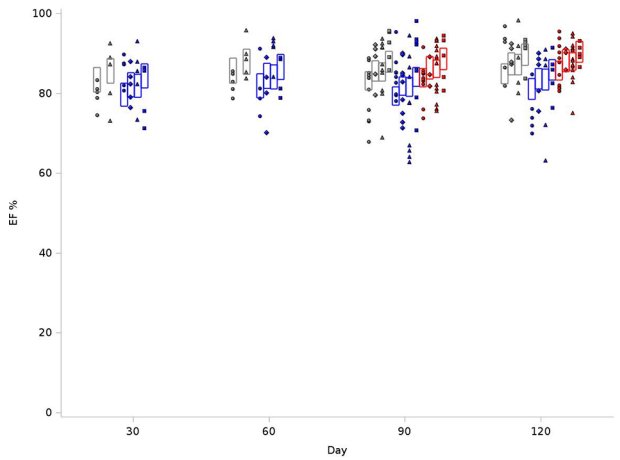


FIGURE 3 | Graphical representation of values for Ejection Fraction (EF) in rats at 4 time points (X-axis). The EFs were measured at 30, 60, 90 and 120 days. The values observed are represented by symbols denoting the experimental treatment: Circle (No Radiation and No Lisinopril -R-L), Diamond (No Radiation and Lisinopril -R+L), Triangle (Radiation and No Lisinopril +R-L), or Square (Radiation and Lisinopril +R+L). The colors represent the strain: Brown-Norway (BN: black), Salt-Sensitive (SS: blue) and SS with BN chromosome 6 (SSBN6: red). The rectangles are 95% Confidence Intervals for EF from a Repeated Measures linear model for treatment that included time and strain. EFs in SS rats were lower than those of other strains (p-value=0.002). They increase with Lisinopril (p-value=0.02) and also with radiotherapy (p-value=0.02).

Figure 4, for the BN control group at 120 days, the average IVSD was 0.83 cm/kg, compared to 0.77 cm/kg in SS control and 0.57cm/kg for SSBN6. Radiotherapy increased the septal wall thickness to 0.92cm/kg, 0.89 cm/kg and 0.66cm/kg, respectively. This represents an 11-16% increase on average in the septal wall thickness for all rats with RT (+0.088 cm/kg, p=0.0001). Lisinopril decreased the IVSD by 12-17% (-0.098 cm/kg, p<0.0001). **Figure 4** demonstrates the changes in IVSD values at 30, 60, 90 and 120 days after radiation.

As outlined in **Figure 5**, for the BN control group, the average LVPWD at 120 days was 0.79 cm/kg, compared to 0.76 cm/kg in SS control and 0.58 cm/kg for SSBN6. There was a 14-15% increase in posterior wall thickness for SS and BN rats who received radiation (+0.11 cm/kg, p<0.0001). For SSBN6 rats, radiation did not increase posterior wall thickness, and in turn demonstrates a decrease of 0.01cm/kg (p=0.0114). Lisinopril reduced LVPWD in all groups, with an average of 11-15% decrease in posterior wall thickness (-0.087 cm/kg, p=0.0003). **Figure 5** demonstrates the changes in LVPWD values at 30, 60, 90 and 120 days after radiation.

Bioenergetics of Mitochondria in PBMCs

Several mitochondrial bioenergetic parameters (described schematically in **Figure 6A**) were measured in PBMCs harvested from a subset of rats at 3 months post-irradiation (sample sizes shown in **Figure 6B**). The 90 day time point was chosen to predict radiation-induced cardiac dysfunction observed at 120 days. At the start of evaluation, basal respiration was measured without the addition of pharmacological inhibitors. Basal mitochondrial respiration was calculated at the end of the experiments as the difference between the oxygen consumption rate in the absence of inhibitors and after addition of Rotenone with Antimycin A, which shuts down the respiratory chain to ablate mitochondrial oxygen consumption. There was no difference in basal respiration or non-mitochondrial respiration between treatment groups from BN, SS or SSBN6 rats (results not shown).

Maximal respiration was measured by addition of the potent uncoupler FCCP. This stimulated oxygen consumption by uncoupling oxidative phosphorylation and disrupting ATP synthesis to freely permit protons to be transported across cell membranes. By subtracting the non-mitochondrial rate of oxygen consumption from this maximal rate of oxygen consumption, the maximal mitochondrial oxygen consumption was derived. Maximal respiration was decreased in PBMC from irradiated SS rats (SS+R-L) as compared to non-irradiated SS rats (SS-R-L) or irradiated SS rats given lisinopril (SS+R+L) (**Figure 6C**). There was no difference in maximal respiration between the other treatment groups from BN, SS or SSBN6 rats (**Figure 6C**).

The difference between the maximal oxygen consumption rate in the presence of FCCP from the basal respiration without inhibitors yielded the spare respiratory capacity of the mitochondria in the PBMCs from each group. Since there

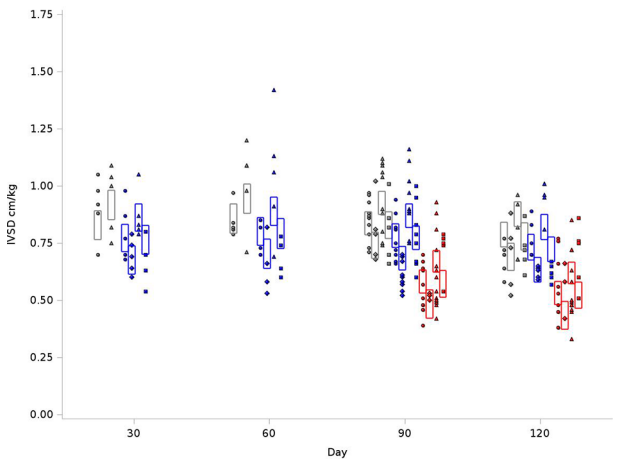


FIGURE 4 | Graphical representation of values for Inter-Ventricular Septum Thickness in Diastole (IVSD) in rats at 4 time points (X-axis). The rats were measured at 4 time points: 30, 60, 90 and 120 days. The values observed are represented by symbols denoting the experimental treatment: Circle (No Radiation and No Lisinopril -R-L), Diamond (No Radiation and Lisinopril -R+L), Triangle (Radiation and No Lisinopril +R-L), or Square (Radiation and Lisinopril +R+L). The colors represent the strain: Brown-Norway (BN: black), Salt-Sensitive (SS: blue) and SS with BN chromosome 6 (SSBN6: red). The rectangles are 95% Confidence Intervals for IVSD from a Repeated Measures linear model for treatment that included time, and strain. IVSD values were normalized to body weight. Radiotherapy increased IVSD by 11-16% at 120 days (p=0.0001) in all rats and lisinopril mitigated this effect by decreasing the IVSD by 12-17% (p < 0.0001).

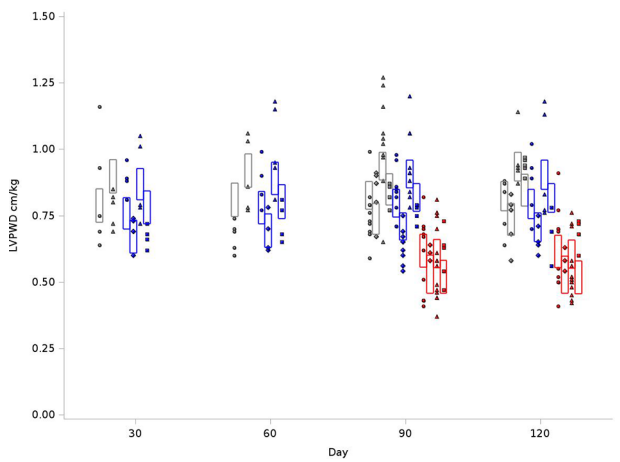


FIGURE 5 | Graphical representation of values for Left Ventricular Posterior Wall Thickness at End Diastole (LVPWD) in rats at 4 time points (X-axis). The rats were measured at 4 time points: 30, 60, 90 and 120 days. The values observed are represented by symbols denoting the experimental treatment: Circle (No Radiation and No Lisinopril -R-L), Diamond (No Radiation and Lisinopril -R+L), Triangle (Radiation and No Lisinopril +R-L), or Square (Radiation and Lisinopril +R+L). The colors represent the strain: Brown-Norway (BN: black), Salt-Sensitive (SS: blue) and SS with BN chromosome 6 (SSBN6: red). The rectangles are 95% Confidence Intervals for LVPWD from a Repeated Measures linear model for treatment that included time, strain and the SSBN6 by radiotherapy interaction. There was 14-15% increase in LVPWD in SS and BN ($p<0.0001$), but not SSBN6 rat hearts at 120 days, while lisinopril reduced LVPWD in all groups of irradiated rat hearts ($p=0.0003$).

was no difference in basal respiration between groups, results were similar to those for maximal respiration (**Figure 6D**). Once again, PBMCs from irradiated SS rats (SS+R-L) had lower spare respiratory capacity than non-irradiated SS rats (SS-R-L) or irradiated SS rats given lisinopril (SS+R+L) (**Figure 6D**).

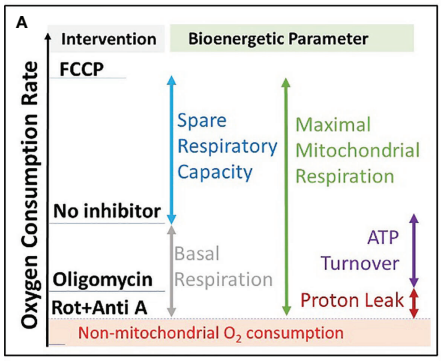
ATP turnover was measured after addition of oligomycin to block mitochondrial ATP synthase. The difference between oxygen consumption rates without inhibitors (at the start of the measurements) and after addition of oligomycin determined the rates of oxygen consumption that contributed to ATP production. The results for ATP production are represented graphically in **Figure 6E**. There was no difference between treatment groups in BN, SS or SSBN6 rats when results were examined by ANOVA. However, PBMCs from irradiated SS rats (SS+R-L) had lower respiration that was coupled to ATP production than from non-irradiated SS rats (SS-R-L) if a comparison by t-test was made between groups inside each strain. The proton leak in the mitochondrial membranes of PBMCs from different rat groups were determined by subtracting the non-mitochondrial respiration from respiration after treatment with oligomycin, which inhibited ATP synthase. There was no difference between treatment groups in BN, SS or SSBN6 rats when results were examined by ANOVA. The proton leak was lower in mitochondria from PBMCs of irradiated SS rats (SS+R-L) than those of non-irradiated SS rats (SS-R-L, **Figure 6F**) if a comparison by t-test was made between groups inside each strain.

DISCUSSION

Small animal models, including rodents, have been used for many decades to study cardiac radiation toxicity, given the physiological similarities that these models have to humans (2, 52, 53). In an effort to understand heritable genetic traits that could modify cardiac radiation sensitivity, Salt-Sensitive (SS) and Brown Norway (BN) strain rats have been used as a cardiac radiation toxicity model (2). A number of different dose and fractionation regimens have been utilized in preclinical studies of RIHD, ranging from large single fractions to more clinically relevant fractionated regimens (2). We used a fractionated regimen consisting of 8 Gy x 5 fractions, similar to prior studies that have utilized fractionated radiation (2, 19, 54) and which have reported histological changes (55, 56).

Looking at the general response of our models to radiation therapy, the cardiac function remained stable, which shows the compensatory mechanisms of the heart. Overall, the systolic function of the animals remained very similar between all 3 strains, which was reflected by the very consistent EF and SV values. The ESV reflects this consistency as the ESV aligns with the changes in EDV. From a contractility and LV function standpoint, the doses of radiation were not overtly cardiotoxic. Nonetheless, radiation induced measurable changes in the hearts of BN, SS and SSBN6 rats. Previous studies by Nabbi et al, 2014 demonstrated cardioprotection in SSBN6 rat hearts that were challenged with ischemia-reperfusion *ex vivo* (27). Such protection was not observed after *in vivo* radiation injury. Perhaps protection may have occurred at later times than 120 days after radiation, which was the longest duration evaluated in the current study.

From a cardiac structure and remodeling perspective, we did observe changes as evidenced by echocardiogram. Prior studies have shown that even partial heart irradiation can produce left ventricular dilation and increased fibrosis in the myocardium and pericardium (16). The linear LV dimensions and the EDV in our BN control rats were within the normal expected range based on previously published results (2, 57). At later timepoints within BN controls, the EDV is reduced. For SS rats, the control animals have a lower EDV which mildly decreases over time. The SSBN6 rat has an unusually low EDV when compared to their parent strains. Despite these differences, radiation reduced EDV in both resistant and sensitive models for radiation induced cardiotoxicity. Changes in EDV are influenced by preload and LV chamber size. In our data, changes in preload, or diastolic filling time, are not evident, given the consistent heart rates between the groups. Therefore, changes in EDV are going to be reflected by some degree of cardiac hypertrophy and reduction in chamber size or compliance. While evaluating changes in LV chamber size, we found that radiation results in increase in both the septal and posterior wall thickness. These changes would be expected if radiation is invoking cellular damage and fibrosis.



B

Group	Abbreviation	Symbol	N
BN non-irradiated	BN-R-L	○	8
BN+lisinopril	BN-R+L	◇	5
BN irradiated	BN+R-L	△	8
BN-R+lisinopril	BN+R+L	□	5
SS non-irradiated	SS-R-L	○	9
SS+lisinopril	SS-R+L	◇	9
SS irradiated	SS+R-L	△	10
SS-R+lisinopril	SS+R+L	□	9
SS-BN6 non-irradiated	SSBN6-R-L	○	4
SS-BN6+lisinopril	SSBN6-R+L	◇	3
SS-BN6 irradiated	SSBN6+R-L	△	4
SS-BN6-R+lisinopril	SSBN6+R+L	□	5

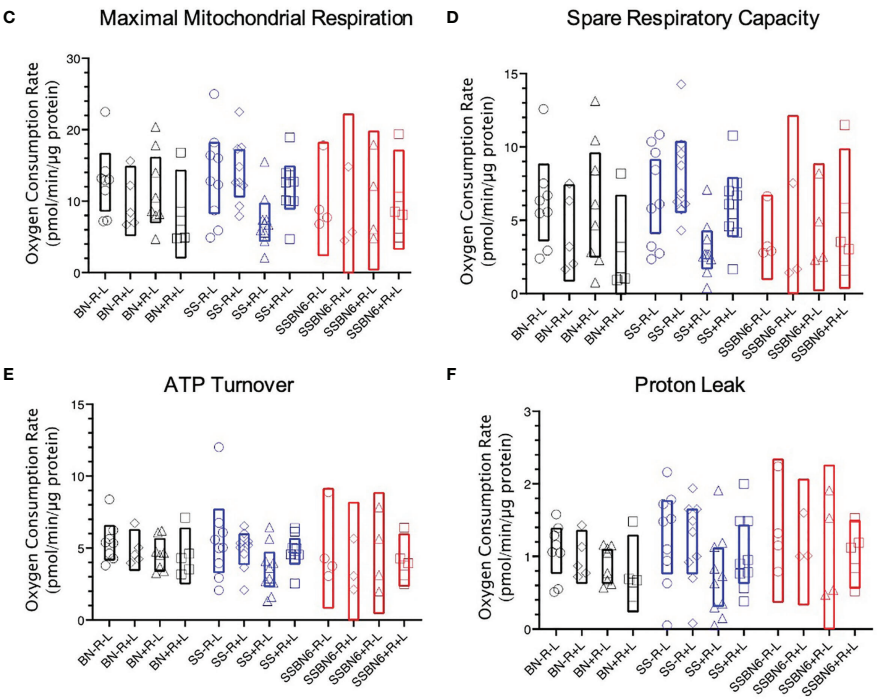


FIGURE 6 | Oxygen consumption rates in peripheral blood mononuclear cells (PBMCs). **(A)** Schematic showing the effects of pharmacological agents on oxygen consumption by PBMCs as investigated by the Seahorse Cell Mitochondrial Stress Test (see *Methods*). If the respiratory chain activity is blocked with Rotenone and Antimycin A (designated as Rot+Anti A) then only non-mitochondrial oxygen consumption remains (shaded and marked in red). The difference between oxygen consumption without an inhibitor and with Rotenone and Antimycin A represents basal mitochondrial respiration (grey bar). Mitochondrial oxygen consumption that is driven by H+ flux through ATP synthase is inhibited by oligomycin. The difference between oxygen consumption without an inhibitor and in the presence of oligomycin gives the oxygen consumption coupled with ATP production (purple bar). The difference between oligomycin-inhibited respiration and non-mitochondrial oxygen consumption gives the proton (H+) leak (maroon bar). The uncoupler FCCP enhances oxygen consumption (blue bar that represents spare respiratory capacity) to yield maximal mitochondrial respiration (green bar). **(B)** Table showing numbers of rats in each group for graphs C-F. Oxygen consumption rates in BN (black), SS (blue) and SSBN6 (red) rats at 90 days post-irradiation. Circles = non-irradiated rats, diamonds = nonirradiated rats given lisinopril, triangles = irradiated rats, squares = irradiated rats given lisinopril. Values are expressed as pmol/minute/microgram protein. **(C)** Maximal mitochondrial respiration. Values were derived as the difference after treatment with the uncoupler FCCP and the non-mitochondrial oxygen consumption rate (represented by green bar in Panel A). FCCP increases the proton flow across the inner mitochondrial membrane creating a H^+ short circuit to maximize oxygen consumption (p=0.020, SS irradiated rats (SS+R-L) versus SS non-irradiated rats (SS-R-L), p=0.040, SS irradiated rats (SS+R-L) versus SS non-irradiated rats treated with lisinopril (SS-R+L)). There was no difference between other groups. **(D)** Spare Respiratory Capacity derived as the difference between treatment with FCCP and followed by subtraction of the basal oxygen consumption rate in the absence of any inhibitor (represented by blue bar in Panel A), (p= 0.019, SS irradiated rats (SS+R-L) versus SS non-irradiated rats (SS-R-L); p=0.023, SS irradiated rats (SS+R-L) versus SS non-irradiated rats treated with lisinopril (SS-R+L)). There was no difference between other groups. **(E)** ATP turnover after treatment with the ATP synthase inhibitor, oligomycin, and subtraction of the basal oxygen respiration values in the absence of any inhibitor (represented by purple bar in Panel A) (p= 0.037 (t -test, not ANOVA) for SS irradiated rats (SS+R-L) versus SS non-irradiated rats (SS-R-L)). There was no difference between other groups. **(F)** Proton (H+) Leak derived by the difference in oligomycin and the non-mitochondrial oxygen consumption rates (represented by maroon bar in Panel **(A)**. Oligomycin inhibits ATP synthase but not uncoupled mitochondrial oxygen consumption from proton leak (p=0.036 (t -test, not ANOVA), SS irradiated rats (SS+R-L) versus SS non-irradiated rats (SS-R-L)). There was no difference between other groups. Values in rats treated with lisinopril were not different from non-irradiated controls for all mitochondrial respiratory parameters represented in **(E, F)**.

The improvement in the linear dimensions (IVSD and LVPWD) with lisinopril reflects the possible anti-remodeling and other actions of this drug to reduce myocyte hypertrophy and fibrosis.

Our results show the effects of radiation to the whole heart, including left posterior wall and interventricular septum hypertrophy and reduction in end diastolic volume. Clinically, patients receiving thoracic RT often receive radiation to only part of the heart, instead of a fairly uniform radiation dose to the whole heart. Preclinical studies which use whole heart irradiation have advanced our knowledge of RIHD, but whole heart radiation might not completely represent the clinical pathophysiology spectrum of RIHD (2, 16, 58–60). Understanding the mechanism of normal tissue radiation injury will help us develop models that more accurately represent the radiation effects observed in patients receiving thoracic irradiation. Furthermore, understanding how to mitigate cardiovascular disease in salt-sensitive populations exposed to radiation has tremendous promise for reducing racial disparities in cancer survivorship between Black and White populations.

This study also examined bioenergetic parameters in PBMCs to determine if these were altered in a manner that would predict cardiac toxicity by radiation. There was no difference in bioenergetics in PBMCs from irradiated versus non-irradiated BN or SSBN6 rats at 90 days, one month before the mild cardiac toxicities were detected in **Figures 2**–**5**. However, mitochondria in irradiated SS rats exhibited lower maximal respiration, and spare respiratory capacity, which was mitigated by lisinopril. ATP turnover and proton leak, though not significant by ANOVA, were lowered by radiation if comparisons were made between all combinations of only 2 groups at a time. Evaluation of the blood cell counts at 90 days in all rats (results not shown) showed no differences in the differential white blood cell counts between irradiated and non-irradiated rats, or irradiated rats given lisinopril. Taken together, these findings warrant further studies into the effects of radiation on the BHI of SS rats and the mitigation by lisinopril. Interestingly, only a partial volume of bone marrow was irradiated in this study, implying that radiotherapy may have a more pronounced effect on mitochondrial respiration in low renin animal models such as SS rats. Bone marrow injury typically recovers by 21 days in irradiated rats after partial body exposures (61) and blood cell counts in the current study were normal at 90 days. Therefore, changes in mitochondrial respiration in these circulating blood cells at 90 days may signal global or abscopal effects of radiation in cells that have not been irradiated. Additionally, there may be a genetic component to such effects since SSBN6 rats appeared more protected from mitochondrial dysfunction than SS rats. Future studies should focus attention on similar data in male rats and on irradiation induced changes in coronary endothelial cells.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by Institutional Animal Care and Use Committee of the Medical College of Wisconsin.

AUTHOR CONTRIBUTIONS

Conceptualization, MM, BF, AB, EJ. Methodology, JN, FG, BF, MZ, TG, DV, MM. Formal Analysis, SO, RS, JN, FG, BF, JO, MM. Original Draft Preparation and Writing, SO, JN, MZ, JO, MM. Review and Editing, SO, RS, JN, NL, FG, BF, MZ, TG, DV, AB, JO, EJ, MM. All authors contributed to the article and approved the submitted version.

FUNDING

Cancer & Cardiovascular Centers, Medical College of Wisconsin, NIAID U01AI133594 and VA Merit Review Award 1I01BX001681.

ACKNOWLEDGMENTS

We would like to thank Leanne Harmann and Kaitlyn Duskin for carrying out the echocardiogram in rats, and Steven Komas and the Cancer Center Redox & Bioenergetics Shared Resource Core for the Seahorse experiments.

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frontiers | Frontiers in Oncology

REVIEW
published: 07 April 2022
doi: 10.3389/fonc.2022.862819

Hypofractionated Whole-Breast Irradiation Focus on Coronary Arteries and Cardiac Toxicity—A Narrative Review

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Breast cancer is the most common cancer among women worldwide, which is often treated with radiotherapy. Whole breast irradiation (WBI) is one of the most common types of irradiation. Hypo-fractionated WBI (HF-WBI) reduces the treatment time from 5 to 3 weeks. Recent radiobiological and clinical evidence recommended the use of HF-WBI regardless of the age or stage of disease, and it is proven that hypo-fractionation is non-inferior to conventional fractionation regimen irradiation. However, some studies report an increased incidence of heart-related deaths in the case of breast irradiation by hypo-fractionation, especially in patients with pre-existing cardiac risk factors at the time of treatment. Due to the new technical possibilities of radiotherapy techniques, HF-WBI can reduce the risk of cardiac toxicity by controlling the doses received both by the heart and by the anatomical structures of the heart. The radiobiological "double trouble", in particular "treble trouble", for hypo-fractionated regimen scan be avoided by improving the methods of heart sparing based on image-guided irradiation (IGRT) and by using respiration control techniques so that late cardiac toxicity is expected to be limited. However, long-term follow-up of patients treated with HF-WBI with modern radiotherapy techniques is necessary considering the progress of systemic therapy, which is associated with long-term survival, and also the cardiac toxicity of new oncological treatments. The still unknown effects of small doses spread in large volumes on lung tissue may increase the risk of second malignancy, but they can also be indirectly involved in the later development of a heart disease. It is also necessary to develop multivariable radiobiological models that include histological, molecular, clinical, and therapeutic parameters to identify risk groups and dosimetric tolerance in order to limit the incidence of late cardiac events. MR-LINAC will be able to offer a new standard for reducing cardiac toxicity in the future, especially in neoadjuvant settings for small tumors.

Keywords: cardiac toxicity, coronary arteries, hypo-fractionated radiotherapy, breast, chemotherapy, HF-WBI

OPEN ACCESS

Edited by:
Virginie Monceau, Institut de Radioprotection et de Sûreté Nucléaire, France

Reviewed by:
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Specialty section:
This article was submitted to Radiation Oncology, a section of the journal Frontiers in Oncology

Received: *26 January 2022*
Accepted: *10 March 2022*
Published: *07 April 2022*

Citation:
Mireştean CC, Iancu RI and Iancu DPT (2022) Hypofractionated Whole-Breast Irradiation Focus on Coronary Arteries and Cardiac Toxicity—A Narrative Review. Front. Oncol. 12:862819. doi: 10.3389/fonc.2022.862819

INTRODUCTION

The risk of cardiac toxicity developed at long intervals after irradiation of the whole breast for early-stage breast cancer in case of breast conservative surgery has been shown to be high, with a potential to cause mortality related to heart disease. Historical studies have shown an increased rate of adverse effects in patients treated for left breast cancer, a fact denied by new scientific evidence. It is considered that the risk of mortality for women treated for left breast cancer is equal to that of women treated for right breast cancer by whole breast radiotherapy (WBI). Hypo-fractionated adjuvant radiotherapy of the whole breast (HF-WBI) is also accepted for the adjuvant treatment of the left breast, with the results indicating a non-inferior loco-regional control and overall survival compared to the case when standard fractionation treatment was used. The HF-WBI is also associated with a 10-year heart rate toxicity equal to that of the control group in which 50 Gy was delivered in 25 daily fractions (1, 2).

Chen and collaborators demonstrated that, for a median follow-up of 14 years, there was no difference in the mortality of cardiac causes in women who were irradiated postoperatively with the conventional or hypo-fractionated regimen on the breast or chest wall. The authors did not report differences in comparative analysis of patient lots irradiated for incipient breast cancer after conservative surgery nor between cases treated for right breast or left breast cancer (3).

Different results have been reported by other authors in some studies, with hypo-fractionated WBI irradiation being associated with an increased risk of late cardiac toxicity. However, the results should be evaluated with caution because some studies reporting an excess of late cardiac events in HF-WBI groups have used conventional techniques and parasternal irradiation, leading to an increase in the doses received by the heart. However, there are factors that may significantly affect the results regarding the toxicities of the treatment and which were not taken into account in most retrospective studies. Thus, pre-existing cardiac diseases and diabetes are factors related to a patient's medical history that seriously influence the late cardiac toxicity rates. Breast size and dosimetric parameters are also not recorded in most studies reporting a higher incidence of cardiac events in the HF-WBI group (4).

HF-WBI IN CLINICAL PRACTICE

HF-WBI and Historical Trials

The use of hypo-fractionation was initially evaluated in four phase III trials; the first of them was initiated in 1986 at the Royal Marsden Hospital and the Gloucestershire Oncology Center. The arm that included hypo-fractionation included 2 regimens (42.9 Gy in 13 fractions and 39 Gy in 13 fractions). The results show an increased rate of local relapses at 10 years in the arm that received 39 Gy in 13 fractions (14.8%), which was 2.7% higher than in the patient lot that received 50 Gy in 25 fractions (12.1%), but the hypo-fractionated regimen of 42 Gy in 13 fractions demonstrated the ability to reduce the rate of loco-regional recurrences (9.6%) at 2.7% compared to standard treatment (5).

A Canadian study by the Ontario Clinical Oncology Group reports differences between late-onset cardiac mortality for WBI-treated patients for the right and left breasts. The hypo-fractionated regimen was 42.56 Gy in 16 fractions, with a 10-year local recurrence rate of 7.5% in the 50-Gy arm and 7.4% in the hypo-fractionation arm and no significant differences in overall survival. The study demonstrates the feasibility of using HF-WBI. A limitation of this study is the use of information from administrative databases that raises suspicions about the correctness of coding and proper registration of risk factors and cause of death. The presence of large numbers of patients with preexisting risk factors associated with late cardiac toxicity could be associated with an increased number of deaths in the arm of patients irradiated with left breast cancer (6, 7).

START A trial was based on the fractionation schemes from the English trial group RMC/GOC, but the dose in one of the 2 arms of hypo-fractionation was reduced to 41.6 Gy. START A was initiated in 1999 with a design similar to the English RMC/GOC trial except for 42.9 Gy. The results were influenced by the use of 10 Gy in 5 fraction boosts in random cases and of the inhomogeneity of the treatment, with 35% receiving chemotherapy and 15% being operated by radical mastectomy. Trial START B was randomized between 1999 and 2001, with 2,215 patients in two groups: standard fractionation and HF-WBI (40 Gy in 15 fractions for 3 weeks), having the same drawback with the use of the 10-Gy boost in 5 fractions and inhomogeneity in the inclusion criteria. The difference between relapse rates at 10 years was 1.4% (3.8 *vs*. 5.2%) in favor of the arm with hypo-fractionated treatment (8). Given the uncertain evidence and the lack of long-term follow-up, the American Society of Radiation Oncology recommends caution in using hypo-fractionation, especially in the sense of avoiding the inclusion of the heart in the radiation field (9).

A study that enrolled 72,134 women diagnosed with breast cancer in 1976–2006 in Denmark and Sweden aimed to evaluate the long-term incidence of radiation-induced cardiovascular disease by comparing the risk of toxicity in the case of right and left breast irradiation. The ratio between the mean heart dose (MHD) for left and right breast irradiation was 2.33, and the highest incidence of cardiovascular events was pericarditis with a ratio of 1.61 and valvular diseases with a ratio of 1.54. Cardiac ischemic disease has been identified as a risk factor affecting toxicity. At the starting time of the historical hypo-fractionation studies, the adjuvant use of transtuzumab was not a therapeutic standard. The potential for cardiac events associated with anti-HER2 therapy is well known. At the same time, the increased life expectancy of patients treated with transtuzumab also increases the risk of cardiac death (10).

With a median follow-up of 13.2 years, a study that included 485 women treated by conventional fractionation whole breast irradiation (CF-WBI) and 5,334 women treated by HF-WBI under the age of 80 years found a difference in hospitalizations between the two groups of patients. Using a competing risk approach, the authors evaluated the cumulative cardiac

morbidity of 10 and 15 years from radiotherapy for patients who received treatment on the right breast or the left breast. However, the inclusion criteria of the lots were not identical in the 2 arms. In addition to the fact that the group receiving CF-WBI included fewer mastectomy patients, it was more common in the HF-WBI group. In the CF-WBI patients, there was a higher prevalence of diabetes. The study does not reveal any difference in cardiac morbidity leading to hospitalization of cardiac causes between the 2 groups at 15 years of follow-up (11).

HF-WBI—Risk Factors and Toxicity Profile

The patients' preferences regarding the choice of conventional fractional or hypo-fractionated radiotherapy after breast-conserving surgery were investigated after a discussion with the oncologist. In total, 348 patients were included in a study, 259 patients of which were being treated by CF-WBI and 89 patients by HF-WBI. The investigators' option was to offer HF-WBI to older patients who did not receive any adjuvant chemotherapy. The study focused only on evaluating acute toxicities considered similar in the two groups except breast pain. This was reported as being perceived as grade 2 only in the CF-WBI group. The authors recommend the use of hypo-fractionation as a method based on these data, the patients' preference, and the lack of acute side effects (12).

The use of adjuvant radiotherapy concomitantly with anti-HER therapy for positive human breast cancer growth factor (HER2) is common, although data evaluating cardiac toxicity are relatively limited if irradiation is co-administered with trastuzumab in adjuvant settings. Data regarding the use of trastuzumab concurrently with HF-WBI are limited in HER2-positive breast cancer. Sayan et al. evaluated acute cardio-toxicity in HER2-positive breast cancer patients who received concurrent CF-WBI or HF-WBI radiotherapy by evaluating the left ventricular ejection fraction (LVEF). Cardiac toxicity was considered if an absolute decrease of LVEF was observed to be ≥10% below the lower limit of the normal value or ≥16% of the baseline value. The study concluded that there is no difference for decreasing the ratio of ejection fraction between the two groups, the median follow-up being 32 months (range, 13–90 months). The study included 100and 41 cases treated by CF- WBI and HF-WBI, respectively. With 7% reduction rate (LVEF) and without cases of congestive heart failure, the authors recommend a long-term evaluation of HF-WBI cardio-toxicity co-administered with transtuzumab. Even though in this study computer tomography (CT) simulation was used for all patients and the technique of blocked inspiration was used to reduce doses to the heart, the non-uniformity of the inclusion criteria was caused by the random use of boost in some patients, at the clinician's decision. In a study evaluating the effect of the combination of hypo-fractionated irradiation and anti-HER2 treatment with transtuzumab, an asymptomatically significant rate of LVEF decline was found to be 7 and 5%, respectively, in groups of patients who received hypo-fractionated radiotherapy and conventional regimen. A variation of ≥10% from normal or ≥16% was considered significant cardiac impairment. The authors consider the difference between the rates of asymptomatic LVEF between the groups that received hypo-fractionated and conventional irradiation to be insignificant (13, 14).

HF-WBI is often used with caution in patients with large breasts, with an extended tumor bed, or for patients who have received chemotherapy due to the risk of possible late cosmetic effects of hypo-fractionation, probably determined by dose heterogeneity. Pectus excavatum or other congenital or acquired abnormalities of the rib cage and sternum alter the geometry of the thorax, and implicitly, the dosimetry may be associated with an increase in the dose received by the heart. Cardiomegaly involves not only an increase in the value of the cardiothoracic ratio above 0.5 but also the anatomical variations that increase the risk of the heart to be included in the irradiation field, not only being a potentiating factor of a possible cardiac toxicity but also a risk factor. As a pre-existing disease, there should be justifying caution in choosing hypo-fractionated radiotherapy. Patel and collaborators evaluated the effect of HF-WBI on patients with large breast volume, including 505 cases of which 502 were treated with HF-WBI, all with whole-breast clinical target volumes of ≥1,000 cm^3. Using a 42.56-Gy hypo-fractionation scheme in 16 fractions in most cases plus a cavity boost of 10 Gy in 4 fractions, delivered both by three-dimensional conformal radiotherapy field-in-field technique and by intensity-modulated radiation therapy technique, the rate of acute grades 1, 2, and 3 dermatitis was 55.0, 40.8, and 3.4%, respectively. Three parameters were identified as predictors of severe dermatitis (a whole-breast clinical target volume of ≥1,500 cm^3, body mass index ≥34, and V105 >10%). The authors recommend maintaining V105 <10% to maintain a grade 3 dermatitis rate of <2% when using hypo-fractionation for patients with large breasts. Using a spectrophotometric method, a study that included 24 patients identified primarily lighter skin color, breast size (small breasts), use of boost, and VMAT technique as risk factors associated with the risk of mild radiation-induced dermatitis. In order to evaluate the safety of the method, a randomized, multicenter study compared the irradiation with conventional CF-WBI fractionation in a total dose of 50 Gy/25 fractions + 10 to 14 Gy/5 to 7 fractions administered as a boost, with HF-WBI in a total dose of 42.56 Gy/16 fractions + 10 to 12.5 Gy/4 to 5 fractions for the boost. The study also showed a cosmetically favorable toxicity profile in cases that did not receive chemotherapy and only a lower increase in skin toxicity if hypo-fractionation was associated with chemotherapy. The authors strongly recommend the use of HF-WBI (13–17).

Without being the subject of this study, however, it should be noted that most trials evaluating the HF-WBI toxicity profile relate to cosmetic effects, especially acute dermatitis (17–20).

Evaluating a randomized unblinded interventional trial CF-WBI (50 Gy in 25 fractions for 5 weeks with a sequential boost of 10–14 Gy delivered in 5–7 fractions) and HF-WBI (46.56 Gy in 16 fractions for a week and a half plus a boost of 10–12.50 Gy in 4 to 5 fractions) followed by conservative breast surgery that included 287 cases with vastness greater than or equal to 40 years and TMN stage between 0 and II, Shaitelman and collaborators consider, after an evaluation of the data at 3 years after treatment, that HF-WBI is feasible both in terms of

results and toxicity profile and large breasts. Chemotherapy and tumor bed boost are not factors that contraindicate hypo-fractionation (18, 19).

Another study that evaluated acute toxicities in a group of 140 patients with breast cancer who received adjuvant radiotherapy with both conventional fractionation and moderate hypo-fractionation showed much lower rates of acute dermatitis as assessed both by evaluation and reporting by the physician and patient, respectively, as well as by spectrophotometric evaluation in the case of HF-WBI. Not only were lower rates of acute dermatitis associated with hypo-fractionated radiotherapy but also hyperpigmentation and limitations of day-to-day activities were lower in the group of patients receiving HF-WBI (20).

THE INFLUENCE TO CARDIAC AND CORONARY ARTERY TOXICITY OF SYSTEMIC ONCOLOGICAL TREATMENT

The cardiac toxicity associated with the systemic treatments administered in the multidisciplinary treatment of breast cancer is well known, which is being associated with both conventional chemotherapy agents and with new innovative target therapies. All these treatments can cause different degrees of cardiac dysfunction. From a dose–effect relationship, type I cardiac toxicity is dose dependent and irreversible, and dose-independent cardiac toxicity is generally reversible. Pertuzumab, a humanized antibody targeting extracellular domain II of HER2, is currently combined with trastuzumab plus docetaxel, a standard first-line treatment in HER2-positive metastatic breast cancer, but the APHINITY trial confirms the value of adding transtuzumab for treatment the invasive HER2-positive early-stage breast cancer. In a total study group of 4,805, including both patients who received pertuzumab or placebo with a median follow-up of 74 months, they did not show elevated rates of cardiac adverse events, with their rate being maintained at <1%. It should be noted that the clinical benefit for pertuzumab addition was only obtained in patients with positive nodes (21).

The absence of changes in troponin-I (TnI) and brain natriuretic peptide (BNP) biomarkers in double-blocked HER2 in combination with taxanes demonstrates the safety of this treatment for cardiac toxicity in the absence of anthracycline therapy, confirming the safety of the transtuzumab–pertuzumab combination in the CLEOPATRA trial result. The final results of the CLEOPATRA trial showed only 1% rate of fatal treatment-related adverse events in the group receiving pertuzumab and 2% in the placebo group for a median follow-up of 8 years. There is 1 new case of congestive heart failure in the pertuzumab group (22–24).

Only one case of asymptomatic decrease in the left ventricular ejection fraction below 50% was identified in a batch of 23 cases of metastatic or recurrent breast cancer HER2+ who received treatment with radiotherapy for treatment with or for palliative care in combination with transtuzumab and pertuzumab. Concomitant dual HER2 blockade (pertuzumab and trastuzumab) with concurrent curative breast radiotherapy was not associated with cardiac toxicity in a study including 55 patients with a median follow-up of 4.1 years. The mean radiation dose was 50 Gy, and the mean decrease in left ventricular ejection fraction was -2.43% before and after chemotherapy and radiotherapy. However, Ito and collaborators report 2 cases in which dual HER2 blockade was associated with decreased ventricular ejection fraction stage in 2 Japanese patients aged 72 and 49 years after 6 and 13 cycles of transtuzumab, respectively (25–27).

The toxicity induced by anthracycline chemotherapy may be acute, manifested im-mediately after infusion of the drug or later, *i.e.*, manifested at varying intervals from 1 to 10 years after treatment. From a clinical point of view, the toxicity of anthracyclines is manifested by thinning off of the wall of the left ventricle, with a gradual decrease of the ejection fraction of the left ventricle (28).

The observation of the cardiotoxicity of anthracyclines in children manifested by the onset of cardiomyopathy led to the conclusion that the cardiac impairment is determined by the death of the cardiomyocyte progenitor cells by apoptosis. A cumulative dose of 400 mg/m^2 is associated with 3–5% risk of heart failure, with the risk increasing up to 26% at doses of 550 mg/m^2 in the case of doxorubicin. The risk of toxicity is lower when liposomal doxorubicin and epirubicin are used. A history of radiation therapy and the concomitant use of cyclophosphamide, paclitaxel, and transtuzumab increase the risk of heart failure. Young and old age and pre-existing cardiac diseases are also factors with the potential to increase the rate of adverse effects (29, 30).

Alkylating agents, including cyclophosphamide, are associated with increased cardio-toxicity, the risk of heart failure being 7–28% at a dose of 150 mg/kg and 1.5 g/m^2. The most common clinical manifestation is pericardial effusion, and the risk is increased for mediastinal irradiation and anthracycline treatment. An ifosfamide dosage >12.5 g/m^2 is also associated with cardio-toxicity (31).

Antimicrotubic agents were associated with 1.7–5% risk of myocardial ischemia in the case of docetaxel and paclitaxel administration, respectively. Bradycardia without clinical significance in most cases is associated with a hypersensitivity reaction following histamine release. Paclitaxel also increases the risk of arrhythmias (32, 33).

Transtuzumab is associated with cardiomyopathy in 2–7% cases, with the potential for cardiotoxicity being increased to 13% risk if paclitaxel treatment is associated. The risk of heart disease can reach 27% if the treatment with anthracyclines is combined. The cardiac toxic effect of transtuzumab is not dose dependent but is strongly amplified by the association with anthracyclines that induce myocyte apoptosis. Studies in mice have shown a synergistic effect with potential for biventricular involvement of both the left ventricle and the right ventricle if the combination of doxorubicin with transtuzumab is administered (34).

A study that included 727 patients treated with anthracycline-based chemotherapy and taxanes and whole breast adjuvant 42.4

Gy radiotherapy in 16 daily fractions (2.65 Gy per fraction) evaluated the effect of transtuzumab addition regarding the degree ≥2 acute skin toxicity and cardiac toxicity, specifically LVEF. The results show a grade 1 (FEVS <60–50%) toxicity rate of 6.8% for patients treated with chemotherapy alone and 13.7% if transtuzumab was added to the regimen. In the case of grade 2 cardiac toxicity (LVEF <50–40%), the rate of toxicity was 1.1% *vs*. 2%, which was higher if transtuzumab was added to hypofractionated radiotherapy and chemotherapy. The authors note that acute cutaneous toxicity was not significantly higher with the addition of transtuzumab. A possible inhomogeneity of the inclusion criteria should also be mentioned; only patients with close or positive margins and those with grade 3 histological tumors received a radiation boost. The authors recommend cautionary measures regarding cardiac toxicity given the increased rate of cardiac toxicity ≥2 degrees in the group of patients who also had transtuzumab included in the therapeutic protocol but still considered transtuzumab with hypo-RT as well tolerated in breast cancer patients (35).

The relationship between cardiovascular effects and the risk of radiotherapy-related cardiac damage was observed both in the survivors of the atomic bomb and in patients who received radiation therapy for the treatment of peptic ulcer, with the risk of cardiac death being increased at >10 years after treatment. Irradiation produces microvascular damage in the case of myocardium and macrovascular damage of the coronary arteries. If in the case of the myocardium the effect appears at a relatively short interval from months to years but often remains asymptomatic due to the possibility of the myocardium to ensure its vascularization and the compensatory reaction to the reduction of the number of viable myocytes is fibrosis and hypertrophy, in the case of coronary artery disease, it is a late-onset effect, clinically manifested after years or decades of radiotherapy treatment. In the case of large vessels, irradiation causes pathological changes of the endothelium and triggers accelerated atherosclerosis. Epidemiological studies that evaluated cardiac mortality in patients treated with mediastinal radiotherapy for Hodgkin's lymphoma revealed 2.7-fold increase in cardiac mortality, with the risk being increased by a radiation treatment history at a young age (36).

DOSE VOLUME CARDIAC AND CORONARY TOXICITY— RADIOBIOLOGICAL CONSIDERATION

Historical clinical observations have shown high rates of cardiac mortality in patients irradiated by conventional radiation therapy for lymphoma in the mediastinum region. A higher mortality rate from causes other than those related to cancer has also been reported in old lots of patients who have been irradiated by conventional radiotherapy. In 1994, Cuzik et al. report, after analyzing data since 1975, that at that time the high mortality rate of all causes in 10-year survivors of breast cancer treatment was not significant, although there was a numerical difference in favor of untreated patients, with an excess of deaths occurring in older historical studies. The authors conclude that reducing the dose received by the heart is essential in reducing the cardiac toxicity of radiotherapy. In 1991, Emami and collaborators formulated the first recommendations based on the toxicity risk dependence according to the dose–volume parameters (48, 49).

Dosimetric recommendations were reviewed in the Quantitative Analyses of Normal Tissue Effects in the Clinic (QUANTEC) guidelines, in which retrospective studies involving more patients treated by 3D-conformal radiotherapy were analyzed. It was also mentioned that radiation therapy for Hodgkin's lymphoma and breast cancer is associated with a much higher rate of cardiac mortality. The QUANTEC guidelines, although not capable of solving the problem of dose–volume constraints, offer some directions and dosimetric constraints that are respected in many radiotherapy centers.

The Quantec guidelines estimate a probability of <1% of cardiac mortality at 15 years for V25 <10% but also mentions the factors that increase the risk of synergistic toxic cardiac effects, especially when using a multimodal approach, including doxorubicin chemotherapy. The authors' recommendation is to intensify research into the elucidation of the mechanisms and radiation doses associated with the increased rate of cardiac events (37).

Currently, all predictions for the risk of pericarditis associated with radiotherapy are based on the radiation dose delivered to the heart. The contouring guidelines do not define pericardium as an organ at risk (OAR), and dose recommendations in order to reduce the risk of pericarditis are not established. The dose conformation obtained by conventional radiotherapy with tangential fields makes the pericardium "a collateral victim of irradiation", being the closest cardiac substructure in the vicinity of the treated target volume. Due to the steep dose gradient behind the target, the pericardium near the rib cage will receive a dose much higher than the rest of the heart. With the introduction of modern techniques of radiotherapy with modulated intensity that have been adopted in breast cancer, the dose distribution has been changed essentially. The current tendency is not only to delimit the whole heart as an OAR but also to delineate its anatomical sub-structures. Since 2010, Feng et al. published an atlas of the heart and cardiac substructure contouring. Douaneet and collaborators proposed 7 years later another atlas that allows the contouring of 15 different cardiac anatomical structures. The use of these atlases in future studies will facilitate the evaluation of dose–volume constraints with clinical toxicity potential. Most of the attention is focused on the coronary arteries and the heart chambers, but there are no recommendations regarding the contouring of the pericardium in atlases of heart contouring. Although radiation-induced pericarditis is rare in the age of modern radiotherapy techniques, it is often underdiagnosed, and the delineation of the pericardium as a risk structure and the identification of dosimetric constraints are necessary in this case, taking into account the unpredictable dose distribution associated with inverse planning techniques (38, 50, 51).

The advantage of modern techniques is brought by the image guidance of the treatment through the concept of IGRT based on the use of standard computer tomography (CT) simulation and the support of on-board imaging devices to perform precise positioning and to improve the ballistic accuracy of the irradiation beam. The administration of radiotherapy using the deep-inspiration breath-hold (DIBH) technique allows the heart to move downward and posteriorly in relation to the target volume of the breast or chest wall, thus increasing the degree of heart sparing (39, 51–55).

The variation of the doses received by the heart from one case to another is an unsolved problem in breast cancer radiotherapy, and the MHD is often used by the surrogate in the therapeutic decision and validation of a treatment plan. However, MHD may have similar values for a case when the whole heart is receiving a low homogeneous dose or a case when the pericardium in the vicinity of the target volume receives a high dose and the rest of the heart receives a lower dose of radiation. Of course, the adverse effects will be different in the two cases, and MHD cannot differentiate between these situations. The BACCART study by Jacob et al. highlights that MHD is not a sufficient dosimetric parameter to predict the individual dose received by the left ventricle and by the coronary arteries, especially to the left anterior descending coronary artery. MHD is not a sufficiently surrogate dosimetric parameter to estimate the risk of cardiac toxicity, with the results showing that although an MHD value <3 Gy was obtained, for 56% of patients treated for left breast cancer, the left anterior descending coronary artery (LADCA) could receive at doses >40 Gy. Dosimetric recommendations are mostly based on expert opinions and are still not sufficiently documented in order to make a prediction of radiation dose distribution for each case and implicitly of the cardiac toxicity risk. When choosing to use altered fractionation such as hypo-fractionation, the equivalent tumoricid dose and also the toxic dose received by the organs from vicinity of target volumes are approximated using the biological effective dose (BED) and can be calculated using the conventional linear–quadratic (LQ) model, which is considered accurate in estimating equivalent doses in case of dose values per fraction <10 Gy. BED differs for different α/β ratios due to tissue heterogeneity, being approximated with the value of 10 Gy for tumors and with the value of 3 Gy for healthy tissues. In reality, α/β differs significantly both for different histological types of tumors and for healthy tissues. In the case of the breast, α/β is considered approximately equal to 4 Gy, which justifies, from the radiobiological point of view, the use of hypo-fractionation. Tumor radio-sensitivity varies widely from case to case between patients who have the same histological type and the same tumor stage. Some authors recommend the use of isoeffect formula for biological effect estimation, assigning values $\alpha/\beta = 2$, 3.5, and 5 Gy, respectively. The hot spots in the irradiated field become "hotter" as the physical radiation dose is transformed into BED, an effect called the "double trouble". Thus, a more careful approach to treatment plans from the point of view of eliminating hot spots is needed both from the target volume and especially from healthy tissues. In order to evaluate the doses received by the heart and its structures, it is necessary to convert the actual doses into the dose equivalent to the radiation dose given in 2 Gy/fraction (EQD2) (40–43).

In the case of hypo-fractionated radiotherapy, a cause for concern is the effect generated by higher total doses associated with hot spots (higher doses per fraction partial volumes). Described as a risk factor for possible toxicities in conventional radiotherapy under the name of "double trouble", the effect is associated with a much higher toxic risk in case of association with hypo-fractionation. In this case, the effect is known as "treble trouble". More than 15 years ago, Jones and collaborators have proposed an adaptation of the LQ model, considered to be an ideal mathematical model, for various clinical situations in which tissue radio-sensitivity can be altered. The model proposed the addition of a BED equivalent or a mean dose-modifying factor (x) for each clinical and therapeutic feature that could decrease tissue tolerance to irradiation. Thus, in the case of adjuvant radiotherapy for breast cancer, *x*-value is estimated to 1,063 for subcutaneous fibrosis for cyclophosphamide, methotrexate, and fluorouracil (CMF) chemotherapy and radiotherapy and 1,033 for shoulder fibrosis in patients >60 years old and receiving lymph nodes. Equivalent to the effect in BED, CMF chemotherapy, surgery, and age were equated as effect with the addition of BED with 6.48, 17.73, and 3.61 Gy, respectively.

The hypo-fractionation of postoperative radiotherapy for breast cancer has been evaluated in a number of large randomized clinical trials, but concerns remain regarding late cardiac toxicity. A study evaluated the predictive ability of the LQ formalism for four evidence-based hypo-fractionation regimes regarding the dose received by the heart. Analyzing the treatment plans for 60 left breast cancer patients treated with WBI tangential fields, Appelt and collaborators corrected the doses to EQD2 using the LQ radiobiological model for five different fractionation schemes, (one conventional regimen and four hypo-fractionation regimens) using different values of α/β. The results of the radiobiological evaluation prove favorable results on hypo-fractionation for $\alpha/\beta = 3$ Gy for the 40 Gy/15 fractions, 39 Gy/13 fractions, and 42.5 Gy/16 fractions regimes. The authors specify that if the heart can be protected by the dose prescribed for the tumor, the heart receives a lower dose even if it is considered $\alpha/\beta = 1$ Gy. The 41.6 Gy in 13 daily fractions regimen does not achieve favorable results regarding heart sparing compared to the standard 50 Gy in 25 fractionation schemes. A study that includes 40 patients with early stages (T1/T2 + N0) of the left breast evaluated the effect of radiation therapy doses on the coronary arteries and heart in the case of adjuvant irradiation after a conservative breast surgery. The radiation doses received by the coronary arteries—LADCA, left circumflex coronary artery (LCx), whole heart, and heart cavities —right ventricle (RV), and left ventricle (LV) were delineated and subsequently dosimetrically evaluated. For all structures, the median dose and the average dose, V5 and V25, were evaluated. The authors identified LADCA as the structure that receives the highest average dose (24.02 ± 8.38 Gy) and proposed balancing the dose constraints between the different structures as a strategy

to limit radiation-induced cardiac toxicity (44–47) Dose constraints for whole heart and heart anatomical structures for breast cancer radiotherapy recommended in clinical practice have been summarized in **Table 1**.

ULTRA-HYPOFRACTIONATED RADIOTHERAPY: HEADING TOWARDS A NEW STANDARD?

A multicenter, non-inferior, randomized, phase 3 trial compared the hypo-fractionation regimen proposed by the FAST-Forward trial (40 Gy in 15 fractions over 3 weeks) with 2 "ultra-hypo-fractionated" regimens regarding efficacy and toxic late effects, both evaluated at 5 years after treatment. Including 4,096 patients from 97 hospitals aged at least 18 years with invasive breast cancer (pT1-3, pN0-1, and M0) and treated with conservative surgery or mastectomy followed by radiotherapy, the phase III trial proposed 2 ultra-hypo-fractionated regimens (26Gy in 5 fractions and 27Gy in 5 fractions). A relative difference was found from the proposed FAST-Forward trial regimen of -0.3 and -0.7% for the treatment regimen of 27 Gy in 5 fractions and 26 Gy in 5 fractions, respectively, regarding the 5-year ipsilateral breast cancer relapse. Both moderate or marked clinically assessed toxicity and photographic evaluation of the breast were associated with a higher risk of late effects associated with the 27-Gy hypo-fractionation regimen in 5 fractions. The authors propose the ultra-hypo-fractionation scheme of 26 Gy in 5 fractions as a safe alternative to the moderate hypo-fractionation with 40 Gy in 15 fractions. After two more trials (Chinese and Danish) that consolidate evidence favorable to moderate hypo-fractionation, the results of the British trial proposing ultra-hypo-fractionation made Rodin and collaborators conclude that "standard" fractionation for is no longer the standard treatment for breast cancer. The Chinese trial, which included 734 patients, confirms that a moderate hypo-fractionated pattern of 43.5 Gy in 15 fractions over 3 weeks plus 8.7 Gy delivered as a boost is not inferior to the standard fractionation regimen plus a 10-Gy boost administered in 1 week. With a recurrence rate in the group with moderate hypo-fractionation of 1.2 *versus* 2% in the group with conventional fractionation and a similar rate of late toxicity in the two groups, the study achieves its goal. In the case of 2 to 3 degrees of acute skin toxicity, hypo-fractionation demonstrates not only non-inferiority but also an obvious benefit with a median follow-up of 73.5 months. The DBCG HYPO trial, a randomized phase III trial that included 882 patients >40 years of age who underwent conservative surgery for ductal carcinoma *in situ* (DCIS) and negative breast cancer nodes, demonstrated both non-inferiority of the moderate hypo-fractionation regimen (40 Gy in 15 fractions) regarding the risk of recurrence at 9 years and similar or even lower values of toxicity and risk of breast induration, compared with the results of the standard fractionation regimen (56–59).

Leap and Kirova point out the need to adjust the doses received by the heart and its anatomical structures in the context of the new ultra-hypo-fractionation schemes. Thus, the proposed dose limits for the standard fractionation regimen may be a trap; the doses proposed by the consensus of DEGRO breast cancer experts (MHD <2.5 Gy, mean left ventricle dose <3.0 Gy, V30 Gy for the left anterior descending coronary artery <2%) also requires accurate evaluation in clinical trials that include

TABLE 1 | Dose constraints for whole heart and heart anatomical structures for breast cancer radiotherapy (48–55).

Organ/structure/subvolume	Constraints (dose volume)	Recommendation (guidelines/author)
Heart	1/3 heart <60 Gy	Emami et al.
	2/3 heart <45 Gy	Gagliardi et al. (QUANTEC)
	3/3 heart <40 Gy	Piroth et al.; German Society of Radiation Oncology (DEGRO)
	V25 <10%	RTOG 1005 hypofractionated trial
	MHD <26 Gy	Nielsen MH et al.
	V30 <46%	Danish Breast Cooperative Group (DBCG)
	Dmean <2.5 Gy	Smith et al.; American Society of Radiation Oncology
	Dmean <4 Gy	
	V10 <30% (left breast)	
	V10 <10% (right breast)	
	V20 <5% (left breast)	
	V20 = 0 (right breast)	
	V20 = 10%	
	V40 = 5%	
	Dmax <40 Gy	
	<1 Gy (right breast WBI)	
	<2 Gy (left breast WBI)	
	Use an alpha/beta ratio of 2.5 for the heart	
Left ventricle	Dmean <3 Gy	Piroth et al.
	Dmean <2.5 Gy	DEGRO
	V5 <17%	
	V23 <5%	
Left anterior descending coronary artery	Dmax <20 Gy	DBCG
	Dmean <10 Gy	DEGRO
	V30 <2%	

a 26 Gy/5 fraction regimen to limit the risk of late toxic cardiac effects, in the context of which the exact value of the alpha/beta parameter is unknown for the heart and its anatomical structures (51, 60).

STRATEGIES TO LIMIT HEART DOSE IRRADIATION: DEEP INSPIRATION BREATH HOLD AND PRONE TREATMENT POSITION

The DIBH technique is part of the strategies designed to minimize the radiation dose received by the heart using its distance from the chest wall by inflating the lung. The patient will maintain deep inspiration during the delivery of radiation and thus will limit both movements of the target volume, avoiding irradiation with higher doses, of the heart and especially of LADCA. Comparing dosimetrically normal breath irradiation with the DIBH technique for post-mastectomy left breast radiotherapy, Darapu et al. obtained favorable results for V5, V10, V25, V30, and Dmean of heart and also for the ipsilateral lung in the case of DIHB. The authors also maintain the disadvantages of the technique (it is time consuming and requires patient cooperation, team training, and careful treatment setup). The technique is considered advantageous both in the use of conventional fractionation and in hypo-fractionation or stereotactic irradiation with a single dose for thoracic and upper abdominal tumors (61, 62).

Even if it clearly demonstrates a dosimetric benefit with a supposed effect in reducing the late cardiac toxicity profile, DIBH does not solve the source of uncertainty generated by heart contractions during treatment. A study that included 20 patients evaluated the displacement of LADA using the contouring of this structure as a risk organ based on systolic and diastolic phase using computed tomography-based coronary angiography with retrospective electrocardiographic gating. All cases were evaluated for the DIHB technique. The average displacement to the posterior area of the treatment field was 2.3, 2.6, and 2.3 mm in left–right and anteroposterior direction, respectively. Although the DIHB technique is not associated with a considerable displacement of the heart during contractions, LAD position variations may differ greatly between the systolic and diastolic phases from one patient to another. The authors recommended maintaining a distance of ≥5 mm between the LAD and the irradiation field edge in order to reduce the risk of coronary events associated with radiotherapy (63).

Longer-duration deep inspire breath-hold for a time of up to 3 min using voluntary hyperventilation, high nasal prong flow, or even continuous positive airway pressure could maximize heart protection. However, with the new irradiation techniques, the delivery time of the dose in case of irradiation of the chest wall and nodal levels may require up to 5 min. Identifying a solution to increase the level of heart protection is a challenge for future investigation. DIHB VMAT planning showed a reduction from 5.4 to 3.6 Gy of MHD, and D2% to the heart was reduced from 19.3 to 13.4 Gy compared to VMAT-free breath (FB-VMAT) treatment plans. In the case of LADA, the average value of Dmean was also reduced from 6.9 to 3.9 Gy, and D2% was reduced from 19.5 to 9 Gy by using the DIHB technique associated with VMAT left breast irradiation (64, 65).

The use of prone position *versus* supine position in radiotherapy of the left breast is still controversial in terms of the benefit of this position for heart sparing. A support vector machine algorithm was chosen to identify parameters that would be suggestive of the need for supine position re-planning, with 198 left side breast patients being included in the study. Cases associated with ≤0.1 cc of the heart included in the irradiation field were excluded, with the algorithm being applied only to plans that included >0.1 cc of the heart in the target volumes isodose. Heart orientation, heart–tumor distance, and lung volumes included in the irradiation field were identified as significant parameters to form the basis of an exclusion algorithm regarding the need for a re-simulation CT planning in supine position and also for the acceptance of the treatment plans in prone position. A dosimetric study proposed by Wang et al., including 116 cases of left side breast cancer treated with the DIHB technique, demonstrated the prone position benefit in 61% of cases compared to supine DIHB, with the benefit being greater for high pendulousness and moderately large breasts. Prone DIHB had the best results in terms of heart and lung sparing for left side WBI when the technique was compared with shallow breathing (SB), supine DIBH, and prone SB (42, 66–73).

If in the case of the reduced dose received by the lung the prone position proved to be 100% beneficial, for MHD, in 15% of cases there was a benefit for supine position. However, the results of this historical study, which included 400 patients—60% of whom met the criteria for inclusion in the evaluation, demonstrate the superiority of prone position in heart sparing by reducing the average in-field heart volume by 7.5 cm^3, representing a median reduction of 85.7% (74).

MR-LINAC—STATE-OF-THE-ART TECHNOLOGY FOR REDUCING CARDIAC TOXICITY

Soft tissue contrast obtained with MR-LINAC machines, a hybrid unit that combines magnetic resonance imaging with linear accelerator, has demonstrated the ability to reduce the high doses received by radiosensitive structures, including cardiac anatomical structures. A study that explored the capacity of MR-LINAC evaluated the mean and maximum (0.03 cc) doses for the heart, LADA, and left ventricle and also the volume that receives at least 5 Gy (V5) of the left ventricle. The optimized plans demonstrated the ability of the technique to reduce MHD by up to 2.5 Gy and the left atrial mean dose by up to 1.2 Gy. Mention should also be made of an increase of >10 Gy in 4 cardiac substrates in one case in the evaluated group. All evaluated cardiac structures were able to benefit from a reduction in D0.03cc and mean dose by rescheduling based on 0.35-T MRIs acquired on an MR-LINAC to optimize CT-based plans to limit cardiac toxicity (75).

When MR-LINAC is used for partial breast irradiation (PBI) in neoadjuvant settings, a better delimitation of GTV may result in a reduction in margins and therefore a limitation of toxicity. For *in situ* tumors, the ability of MR-LINAC to delimit/irradiate the gross tumor more precisely is obvious, and a higher reduction of setup errors can be observed in both cases of neoadjuvant and adjuvant settings. Comparatively evaluating the doses received by OARs for PBI planned in prone and supine position using MR-LINAC, Koerkamp et al. identified a benefit in reducing V5Gy to ipsilateral long for prone position, with the MHD results being similar in both positions for treatment (76–78).

CONCLUSIONS

The use of HF-WBI radiotherapy has now become a standard in adjuvant treatment after breast-conservatory surgery, although the preliminary results show the feasibility and technique in terms of benefit in loco-regional control and toxicity studies are needed to evaluate the long-term and very-long-term implications, especially for patients who have been irradiated for left breast cancer. It is also necessary to elucidate the mechanisms of action of the new oncological therapies and the potential synergistic interactions with chemotherapy and radiotherapy. Studies that will include the newly considered radiosensitive anatomical structures of the heart, especially the ventricles and coronary arteries, and will correlate toxicity with the doses received by them and with biological and genetic factors related to the patient and the treatments administered will lead to new dose-volume recommendations and to the development of radiobiological models for estimating the risk of toxicity with clinical applicability. The still unknown effects of small doses spread in large volumes on lung tissue may increase the risk of second malignancy, but they can also be indirectly involved in the later development of a heart disease. The changes in conclusions in historical series will probably be accounted for by changes in radiotherapy technique, *e.g.*, use of cobalt therapy, and improved treatment planning due to CT scanning with lung density correction and use of intensity modulation techniques to improve dose uniformity. These will have reduced cardiac dose exposure. However, on the use of hypo-fractionated and especially ultra-hypo-fractionated techniques, associated with a much richer spectrum in systemic therapies, all in the context of considerable improvements in the prognosis of breast cancer, special attention should be paid in the future to limiting the toxic effects of radiotherapy on the heart and coronary arteries. MR-LINAC will be able to offer a new standard for reducing cardiac toxicity in the future, especially in neoadjuvant settings for small tumors.

AUTHOR CONTRIBUTIONS

All authors contributed to the article and approved the submitted version.

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frontiers | Frontiers in Oncology

ORIGINAL RESEARCH
published: 12 April 2022
doi: 10.3389/fonc.2022.869529

Quantifying Societal Burden of Radiation-Induced Cardiovascular Events in Breast Cancer Survivors

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OPEN ACCESS

Edited by:
Marjan Boerma, University of Arkansas for Medical Sciences, United States

Reviewed by:
Chenghui Li, University of Arkansas for Medical Sciences, United States
Sophie Jacob, Institut de Radioprotection et de Sûreté Nucléaire, France

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Specialty section:
This article was submitted to Radiation Oncology, a section of the journal Frontiers in Oncology

Received: *04 February 2022*
Accepted: *09 March 2022*
Published: *12 April 2022*

Citation:
Kimpe E, Werbrouck A, De Ridder M and Putman K (2022) Quantifying Societal Burden of Radiation-Induced Cardiovascular Events in Breast Cancer Survivors. Front. Oncol. 12:869529. doi: 10.3389/fonc.2022.869529

Background and Purpose: Radiation-induced cardiotoxicity is an important health concern for clinicians during treatment of breast cancer (BC) patients. Underlying mechanisms are well-documented, whereas little is known about the societal impact of this long-term effect. This study aimed to quantify the additional burden of radiation-induced cardiovascular (CV) diseases in BC survivors.

Materials and Methods: Conventional health economic modelling techniques were applied to estimate attributed CV-related costs and disutility in a hypothetical cohort of BC survivors. A situation in which radiotherapy caused an additional CV risk was compared with a situation in which this risk was not taken into account. Uncertainty was assessed *via* deterministic and probabilistic sensitivity analyses. Analyses were performed from a broad societal perspective up until 20 years after BC treatment.

Results: Radiation-induced cardiotoxicity evokes a mean incremental cost of €275.10 per woman over a time horizon of 20 years after BC treatment. An additional decrement of 0.017 QALYs (per woman) might be expected when taking the radiation-induced cardiotoxic risk into account in BC survivors. Incremental costs and disutility increased with age. A scenario analysis showed that these results were more profound in women with more advanced staging.

Conclusion: Our analyses suggest that with current radiation techniques, rather minor costs and disutility are to be expected from radiation-induced cardiotoxicity in BC survivors. The cost of past investments in order to achieve current mean heart dose (MHD) seems justified when considering the gains from cost and disutility reduction resulting from radiation-induced cardiovascular events. The question we might consider is whether future opportunity costs associated with investments on further technological advancements offset the expected marginal benefit from further reducing the MHD.

Keywords: radiotherapy, cardiotoxicity, breast cancer, survivorship, health economics

1 INTRODUCTION

Breast cancer (BC) is the most frequently diagnosed cancer type in women worldwide, accounting for more than one quarter of all newly diagnosed female cancers (1, 2). Early detection of breast cancer is enhanced by nationwide accessibility of screening programs for women at risk (3). As a result, breast cancer is diagnosed more frequently at an early stage, leading to a beneficial prognosis. Concurrently, advances in radiotherapy have led to a significant reduction in local recurrence and BC mortality (4). Radiation is administrated to over half of all BC patients (5), and therefore, it is likely that radiotherapy contributes to high survival rates. In developed countries, such as Belgium, the 5-year relative survival rate is around 91% (6).

However, radiation from radiotherapy is not limited to tumor tissue. Despite the use of state-of-the-art radiation techniques, healthy organs -such as the heart- are exposed to minor radiation, leading to inflammatory responses in this tissue (7). These radiation-induced cardiotoxic effects expose BC survivors to an increased long-term risk for cardiovascular (CV) diseases (7–9). These events may have an additional impact on the health-related quality of life (HRQoL), in particular as HRQoL might already have been influenced by BC treatment prior to a potential CV event (10). Additionally, CV events are associated with an increased CV-related mortality risk (11). A meta-analysis demonstrated that the rate ratio for CV mortality was 1.3 (SE 0.09; 2p=0.0007) in BC patients receiving RT compared to controls undergoing the same treatment without RT. Although this meta-analysis provided evidence on benefits from reduction in breast cancer mortality by RT during treatment, the researchers demonstrated the moderate increase in death from CV events in particular in the first and second decade after treatment (4). Other studies indicated similar results (12). However, these studies mainly addressed CV mortality. Although research indicated that the economic burden of cardiovascular diseases soars compared to the health expenditure for other diseases, estimated around 9% of total health care expenditure across European countries (13), the long-term health outcome and costs of cardiovascular morbidity in breast cancer survivors are less considered. The application of health economic evaluation techniques poses an opportunity in order to model these long-term CV effects as it "*provides a framework to make best use of clinical evidence through an organized consideration of the effects […], health care costs, and other effects that are regarded as valuable*" (17). Formerly, health economics research in the field of oncology have largely focused on costs and effects related to primary BC treatment rather than exclusively on long-term CV mortality and morbidity (14–16).

Most of these health economic studies did not include long-term cardiotoxic costs and effects in their analysis. There is still a lack in the more precise quantification of the societal burden of radiation-induced CV diseases. Our study aims to quantify the additional burden of radiation-induced cardiovascular diseases in BC survivors.

2 MATERIALS AND METHODS

2.1 Study Design and Perspective, Target Population and Setting

We employed decision-analytic modelling techniques in which data from various sources is combined to populate a theoretical model (17). We used two types of cohort models, a decision tree and Markov model, to estimate the proportion of patients that would encounter an event over the intended time horizon (i.e. cardiovascular events and/or death). During each cycle, these proportions are attributed to different health states which relate to costs and disutilities (18). The model simulates a cohort of 39-84 year old women diagnosed with stage 0-3 breast cancer, and who were eligible for curative primary BC treatment with or without radiotherapy. Since radiotherapy is not standard care for women with metastatic cancer (stage 4), they were excluded from the cohort. The administration of chemotherapy was left out because this would not differ between the evaluated alternatives, and therefore, would not influence the reported incremental result. Data originates from various sources (European and American studies, Belgian databases). Therefore, the model utmost applies to populations with similar female BC incidence rates, and adherence to the proposed treatment guidelines on primary BC treatment (19–22). A broad, societal perspective is adopted in this cost-utility analysis (CUA). In this perspective, the term "societal burden" refers to cost and consequences of diseases to society (17). In contrast to a health care payer perspective, a wider range of costs is included in this perspective. Typically, indirect costs generated outside the health care system are included in the analyses (17). Hence, direct medical health care costs (primary care, outpatient care, inpatient care, prehospital E&A care and pharmaceuticals) and indirect health care costs (production loss due to premature death, sick leave and permanent disability) were considered. The study protocol was approved by the ethical committee at the Universitair Ziekenhuis Brussel, Brussels, Belgium (B.U.N. 1432020000259). All analyses were performed in Microsoft Excel® 2016.

2.2 Cycle Length, Time Horizon and Discount Rate

Cardiovascular events are typically emerging as a long-term effect from radiotherapy, starting within the first 5 years up until 30 years after primary treatment (23). A time horizon of 20 years after BC treatment was considered. The cycle length was determined at one year. In accordance to Belgian guidelines, discount rates of 3% on costs and 1.5% on outcomes were applied (24).

2.3 Model and Model Assumptions

In this study, we combined a decision tree and Markov model to model probabilities, expected costs and expected (dis)utilities in order to stratify the cohort based on their exposure to radiotherapy and analyse long-term radiation-induced cardiotoxic effects, respectively. A radiation-oncologist (MDR) was closely involved in the modelling process to validate the assumptions in both models to assure accordance with clinical practice.

2.3.1 Model I: Primary Breast Cancer Treatment (Decision Tree)

The decision tree represents different treatment pathways in primary BC patients (19–22). The different branches result in either receiving radiotherapy or not. For women who received radiotherapy, left- and right-sided tumors were assumed to lead to high or moderate radiation exposure, respectively. No radiation exposure was assumed for women who did not receive radiotherapy. The endpoints served as the starting point to stratify female BC patients in the initial states of the Markov model (**Figure 1A**, for details see **Supplementary Material 1, 2**).

2.3.2 Model II: Long-Term Radiation-Induced Cardiotoxicity (Markov Model)

The initial states were defined as [1] 'high radiotherapy exposure', corresponding with a mean heart dose (MHD) of 3.6 Gy [2], 'moderate radiotherapy exposure', correlating with 1.9 Gy and [3] 'no radiotherapy exposure', assumed for women who did not receive radiotherapy (i.e. 0 Gy), and accounting for the laterality of the tumor. The respective MHD means (i.e. 3.6 Gy and 1.9 Gy) were based on a recent systematic review reporting studies published between January 2014 and September 2017. As a variety of RT techniques (e.g. 3D-CRT, step-and-shoot IMRT, rotational IMRT,…), with target doses between 39.9 Gy and 50.4 Gy were reported (25), we believed the assumed MHD correlated well with characteristics from our cohort.

Starting from the initial states, patients either stay in this state, or transfer to one of the three other states. Firstly, a patient may experience a non-fatal cardiovascular event. Secondly, the patient may also immediately die from the cardiovascular event. Lastly, the patient could also decease from other causes (i.e. fatal non-CV events). The two fatal event states were absorbing states, meaning that patients who entered one of the fatal states could not transition back to the other states (**Figure 1B**).

2.4 Comparators and Analytic Methods

2.4.1 Comparators

In order to quantify the additional burden of radiation-induced CV diseases in BC survivors, we evaluated two alternatives: [1] a situation in which an increased radiation-induced cardiovascular risk was considered, and [2] a comparative situation in which the additional risk of radiotherapy was not taken into account (see **Supplementary Material 3**). This strategy was based on a methodology proposed by Mulliez et al. Their approach consisted of adding an excess risk to the traditional SCORE calculation for women who were exposed to radiotherapy (26).

2.4.2 Analytical Methods

A theoretical cohort of 1,000 female BC survivors aged 39-84 years at diagnosis was assumed. Each age in the cohort was weighted according to Belgian age-specific incidence rates. Expected cost and quality adjusted life years (QALYs) for the two situations were

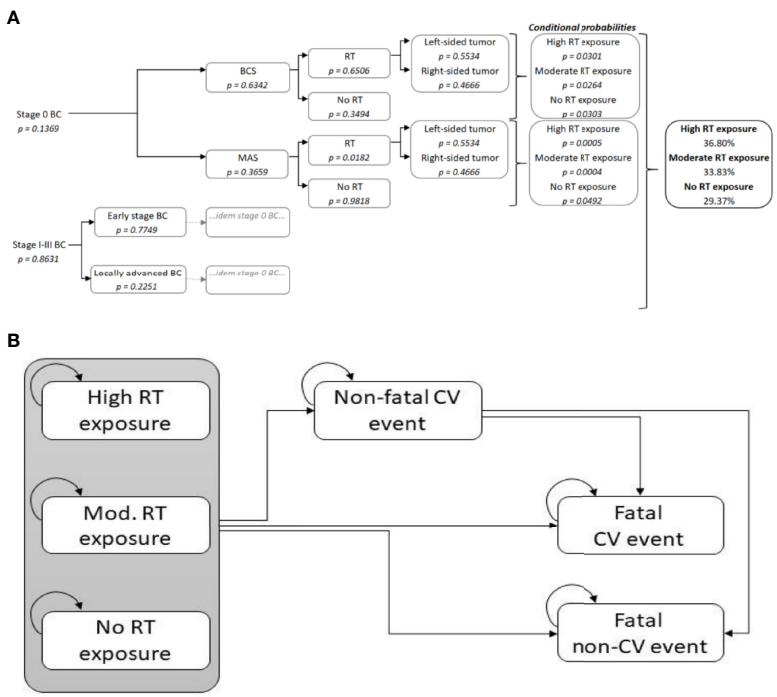

FIGURE 1 | **(A)** Decision tree. The branches represent different primary breast cancer treatment pathways. When radiotherapy is administrated, this results in a radiation dose to the heart according to the laterality of the tumour (for further details see **Supplementary Material 1**), **(B)** Markov model. The boxes represent the six health states of the Markov model. During each yearly cycle, patients may remain in the same state, or transition to another state (represented by the arrows in the figure). BC, Breast Cancer; BCS, Breast Conserving Surgery; CV, Cardiovascular; MAS, Mastectomy; RT, Radiotherapy.

calculated. Typically in a traditional health-economic evaluation, incremental costs and effects would be presented as cost-effectiveness ratios (ICERs). Since a negative health outcome (i.e. less QALYs) was expected in the situation in which the increased radiation-induced CV risk was considered, calculating ICERs was irrelevant. Therefore, results were presented as (mean) incremental costs and (mean) incremental QALYs per woman over a time horizon of 20 years after BC diagnosis.

In the deterministic base case analyses the model was ran based on the most likely assumptions and input parameters (27). Different deterministic scenario analyses were performed. In these scenarios we examined results within specific subgroups by stage and radiation strategy.

In the sensitivity analyses, base case assumptions and input variables were altered in order to assess the uncertainty in the model (27). In a deterministic one-way sensitivity analysis, the most influential parameters were determined by decreasing and increasing each variable separately by an arbitrary chosen proportion of 30% (i.e. 70% and 130% of the base case value). These results were visualized in Tornado diagrams for incremental costs and health outcomes separately. Finally, a probabilistic sensitivity analysis (PSA) was performed *via* Monte Carlo simulations. We ran 1,000 iterations to evaluate the uncertainty around the base case point estimates. Sampling was established by applying distributions which fitted characteristics of the parameters (**Table 1**).

TABLE 1 | Model input parameters (probabilities, costs and utilities).

Parameter	Deterministic value	Distribution	Source
Probabilities and SCORE input variables			
Cohort stratification: high RT exposure	0.3680*		*Decision tree*
Cohort stratification: moderate RT exposure	0.3383*		*Decision tree*
Cohort stratification: no RT exposure	0.2937*		*Decision tree*
Mean heart dose: high RT exposure	3.6000	Log-normal	(25)
Mean heart dose: moderate RT exposure	1.9000		(25)
Mean heart dose: no RT exposure	0.0000		
Calculated ratio MHD high RT/MHD moderate RT	1.8947	Log-normal	
Excess risk ratio for cardiotoxicity	0.0410	Log-normal	(28)
Systolic blood pressure 40-64 years	116.0847	Normal	(29)
Systolic blood pressure 65+ years	136.2094	Normal	(29)
Total cholesterol 40-64 years	5.1832	Normal	(29)
Total cholesterol 65+ years	5.4144	Normal	(29)
Rate of smokers in a cohort of breast cancer survivors	0.0951	Beta	(30)
Probability of fatal CV event in breast cancer survivors+	*0.0427*	*Beta*	(31)
Probability of non-fatal CV event in breast cancer survivors+	*0.1175*	*Beta*	(31)
Calculated risk of non-fatal CV events/fatal CV events+	2.7515		
Probability of fatal CV event after non-fatal CV event	0.0115	Beta	(32)
Probability for fatal non-CV event in breast cancer survivors	0.0181	Beta	(31)
Work-related activity rate for women	0.6490	Gamma	(33)
Probability of permanent disability after non-fatal CV event	0.0394	Beta	(34)
Probability of temporarily sick leave after non-fatal CV event	0.9616		(35)
Mean sick leave days during first year after non-fatal CV event	48.0000	Gamma	(33)
Mean sick leave days in following years after non-fatal CV event	25.0000	Gamma	(33)
Costs**			
Annual wage for women aged 40-65 years, anno 2021	55,559.7500	Gamma	(36)
Daily wage for women aged 40-65 years, anno 2021	252.3000	Gamma	(36)
Primary care costs during first year after non-fatal CV event	54.1200	Gamma	(37)
Primary care costs in following years after non-fatal CV event	94.7100	Gamma	(37)
Outpatient care costs during first year after non-fatal CV event	196.3300	Gamma	(37)
Outpatient care costs in following years after non-fatal CV event	155.7800	Gamma	(37)
Prehospital A&E care costs after non-fatal and fatal CV event	455.6200	Gamma	(38)
Inpatient care costs after non-fatal CV event	4547.5100	Gamma	(39)
Pharmaceutical costs after non-fatal CV event	149.0882	Gamma	(40)
Utilities (QALYs)			
Utility for women in the initial state(s)	0.7700	Beta	(29)
Disutility for chronic CV disorders in Vietnam°	*0.1100*		(41)
Quality of life in Vietnam (all ages)°	*0.9100*	*Log-normal*	(41)
Calculated utility for women in the non-fatal CV event state	0.6769		

Cohort stratification is based on age-specific incidence rates resulting from the decision tree (see **Supplementary Materials 1, 2). The values presented in this table are mean values for all women in the modelled cohort (39-84 year).*

*+For details on all age-specific transition probabilities for non-fatal CV events and fatal CV events, see **Supplementary Material 4**.*

A&E, Accident & Emergency; CV, Cardiovascular; MHD, Mean Heart Dose; RT, Radiotherapy; QALYs, Quality Adjusted Life Years.

*** All costs are converted to and expressed in EUR (€, 2021).*

° Relative disutility was calculated from a Vietnamese cohort with resembling characteristics. This proportional decrease was applied to Belgian female population to calculate 'utility for women in the non-fatal CV event state'.

2.5 Study Parameters

In this model-based analysis various sources were used to populate the two models (**Table 1**). Population-based national data, from the Belgian Cancer Registry (2017), and secondary data retrieved from the literature were used to populate the decision tree (for details see **Supplementary Material 1, 2**). In the Markov model we included secondary data from literature, and data from the Belgian Health Examination Survey, 2018 (29). The risk of cardiovascular mortality was estimated *via* the SCORE equations (42). An excess risk of 4.1% per Gy MHD was considered to account for the radiation effect (26). Detailed information on transition probabilities for fatal and non-fatal CV events is provided in **Supplementary Material 4**. The input variables for the SCORE calculation (i.e. systolic blood pressure and cholesterol) were based on age-specific mean values for Belgian females (29). Smoking status was added as a weighted mean of smokers in a population of BC survivors (30). We used event rates for fatal and non-fatal CV events from a Dutch breast cancer cohort study to calculate the relative risk (RR) for non-fatal CV events, resulting in a RR of 2.75 non-fatal CV events for each fatal CV event (31). Concerning the transition from the non-fatal CV event state to the fatal CV event state, event rates from the EUROASPIRE were applied (32). We established the probability for a fatal non-CV event by excluding cardiovascular event rates from all-cause mortality rates. The latter was retrieved from the same Dutch cohort study that was used to extract non-fatal CV events (31). All probabilities were recalculated to annual transition probabilities (17).

2.6 Costs

An overview of costs is given in **Table 1**. In the analysis of costs, only costs as a result of radiation-induced cardiotoxicity were considered. Costs related to the primary BC treatment were not included, as these were nullified between both alternatives. Direct costs are retrieved from various Belgian and European sources (37–40). Assumptions are based on clinical practice guidelines and -if deemed necessary- validated through expert opinion (MDR). Costs resulting from productivity loss (indirect costs) were based on Belgian wages (33, 36), and calculated using the human capital approach pertaining temporarily or permanent disability and premature death (17). All costs were converted to current prices (2021 euros) with the CCEMPG-EPPI-Centre Cost Converter (43).

2.7 Health Outcomes

QALYs are the preferred measure of health outcome in CUA (17). As with costs, we assumed that the benefit of prolonged survival from radiotherapy would be equal in the two comparative situations as the cohorts received the same primary breast cancer treatment. Research indicates HRQoL in long-term BC survivors is not significantly different from HRQoL in age-matched women in general population (44, 45). Therefore, we used HRQoL from a national survey for women in the initial states (29). The relative disutility for chronic cardiovascular disorders was calculated from a recent comprehensive systematic review (41), which comprised data from a cohort with resembling characteristics (46). The relative disutility was calculated as proportional decrease in HRQoL in the reference population of this study (46). Then, this proportional decrease was applied to the HRQoL in Belgian females (29) and served as basis for the utility of women experiencing a non-fatal CV event. Utilities are summarized in **Table 1**.

3 RESULTS

3.1 Base Case Analyses

Baseline results are presented in **Table 2**, showing expected numbers of non-fatal and fatal CV events per 1,000 women over a time horizon of 20 years after BC treatment. Overall, the situation in which cardiotoxicity was taken into account resulted in more than four additional non-fatal CV events and more than two additional fatal CV events. These extra cases resulted in an average incremental cost of €275.10 per

TABLE 2 | Baseline results in a total cohort of 1,000 female breast cancer survivors[1].

Age at diagnosis	% of the population[c]	Expected number of non-fatal CV events			Expected number of fatal CV events		
		RT risk taken into account[a]	RT risk not taken into account[b]	Δ	RT risk taken into account[a]	RT risk not taken into account[b]	Δ
39-44	6.36%	0.73	0.70	+0.03	0.30	0.29	+0.01
45-49	9.40%	2.08	2.00	+0.08	0.89	0.85	+0.03
50-54	12.39%	6.39	6.15	+0.24	2.61	2.51	+0.10
55-59	12.67%	11.93	11.49	+0.44	4.95	4.77	+0.18
60-64	14.10%	23.71	22.86	+0.84	9.65	9.32	+0.32
65-69	14.26%	36.58	35.36	+1.21	15.11	14.64	+0.46
70-74	12.31%	53.81	52.81	+1.00	18.26	17.77	+0.48
75-79	9.84%	57.91	57.40	+0.51	18.41	18.02	+0.39
80-84	8.67%	61.43	61.46	-0.04	18.22	17.90	+0.31
Total (all ages)	**100%**	**254.56**	**250.24**	**+4.32**	**88.39**	**86.09**	**+2.30**

[1]*Expected number of non-fatal CV events and fatal CV events 20 years after breast cancer diagnosis, comparing the situation in which radiation-induced cardiotoxicity is taken into account (columns a) and the situation in which no additional risk of radiation-induced cardiotoxicity is considered (columns b) during analysis. Results are presented for a total cohort of 1,000 women in which the events are weighted according to their age-specific incidence rates (column c).*

CV, Cardiovascular; RT, Radiotherapy.

woman, and an average incremental utility decrement of 0.017 QALYs per woman. The base case analysis revealed that increasing age is associated with accumulating incremental costs and decreasing incremental QALYs (**Figure 2**). Direct costs appeared to be the main contributors in total costs, being thirteen times higher than indirect costs (€254.16 vs. €20.94).

3.2 Deterministic Scenario Analyses

In the base case analysis, all women eligible for radiotherapy administration after BC diagnosis were considered, regardless of stage (stage 0-3) and RT administration (with or without radiotherapy). **Figure 3** represents a first series of scenario analyses, which demonstrate the effect of the stage at time of

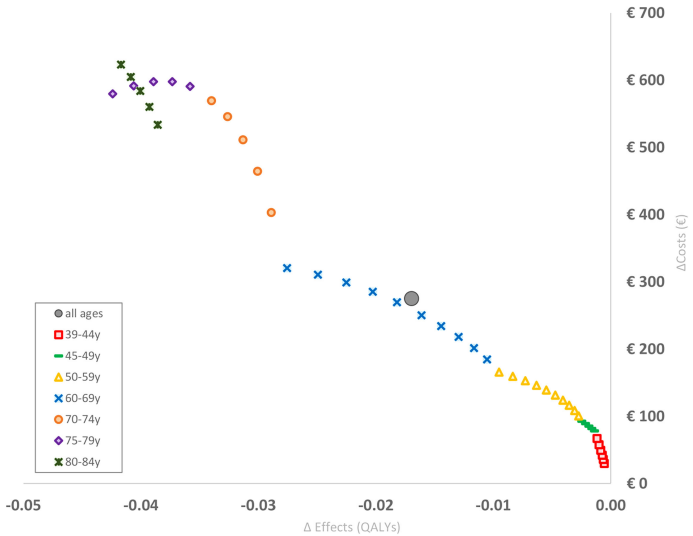

FIGURE 2 | Base case analysis. Each small point estimate represents the incremental cost and disutlity for a respective age (unweighted for age-specific incidence rates) over a time horizon of 20 years after BC diagnosis, and shows the increasing trend with aging. The large point estimate indicates the mean incremental cost and disutility for all women in the cohort (i.e. Δ costs , €275.10 and Δ disutility , 0.017 QALYs). BC, Breast Cancer; QALYs, Quality Adjusted Life Years; y, years.

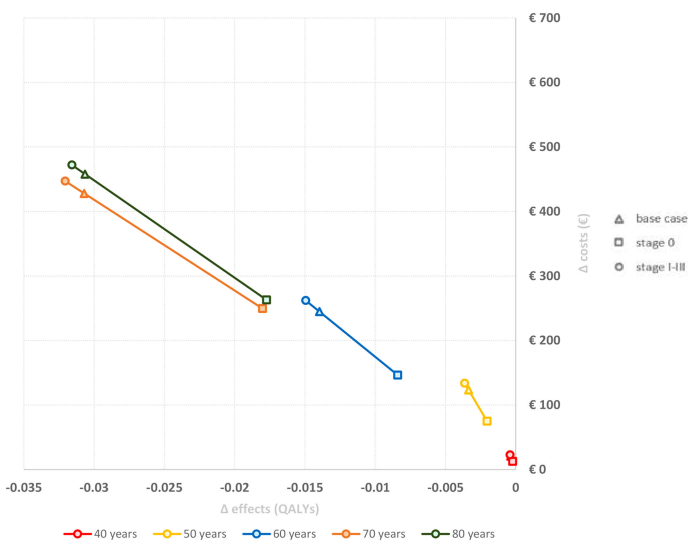

FIGURE 3 | Deterministic scenario analysis: Incremental costs and disutility by stage. The proportions of the start cohort (decision tree) were set to 0% or 100% in order to exclude either stage 0 or stage 1-3 from analysis. This resulted in various initial state cohort stratifications, which is indicated through different symbols. Results are presented as incremental costs and QALYs per woman for four different age groups (40 years, 50 years, 60 years, 70 years and 80 years). BC, Breast Cancer; QALYs, Quality Adjusted Life Years; y, years.

diagnosis. For all ages, a diagnosis of breast cancer at an earlier stage resulted in lower costs and higher QALYs compared to more advanced breast cancers. Radiotherapy is more likely to be omitted in stage 0 BC, resulting in less women in the high and moderate radiotherapy exposure groups, and therefore, explaining this effect. Furthermore, the relative difference became more profound in women diagnosed at older age. A second scenario analysis, showed that base case incremental costs and disutility were underestimated if only women who effectively received RT were included. Incremental costs increased with €109.53, while incremental QALYs aggravated with -0.0067 compared to the base case (Δ costs: €384.63 per woman, Δ QALYs: -0.024 per woman).

3.3 Sensitivity Analyses

One-way sensitivity analysis identified the following parameters as most influential on incremental costs: (1) probability to transition to the 'non-fatal CV event' state from initial state, (2) systolic blood pressure for women aged 65+ years, (3) cost of inpatient care (**Figure 4A**). For incremental QALYs, most affecting parameters were (1) utility for women in initial state, (2) excess risk ratio for cardiotoxicity, (3) cholesterol level for women aged 65+ years (**Figure 4B**).

In the PSA, all iterations -as expected- resulted in higher costs and less QALYs. Consequently, if an additional radiation-induced cardiovascular risk is taken into account in BC survivors in order to estimate long-term costs and outcomes, this will always result in lower health outcomes and higher costs. The analysis showed that the mean value of incremental cost over all iterations was €131 (minimum) and €2,678 (maximum) per woman over a time horizon of 20 years (σ^2 = 54,292.67; σ= 233.01), respectively. Similarly, incremental disutility among the iterations of the average value of all iterations varied between a minimum of -2.727×10^{-4} QALYs and maximum of -0.564 per woman ($\sigma^2 = 2.803^{-3}$; σ= 0.053).

4 DISCUSSION

Our study focused on the incremental costs and effects attributed to radiation-induced cardiotoxicity. The analysis showed that

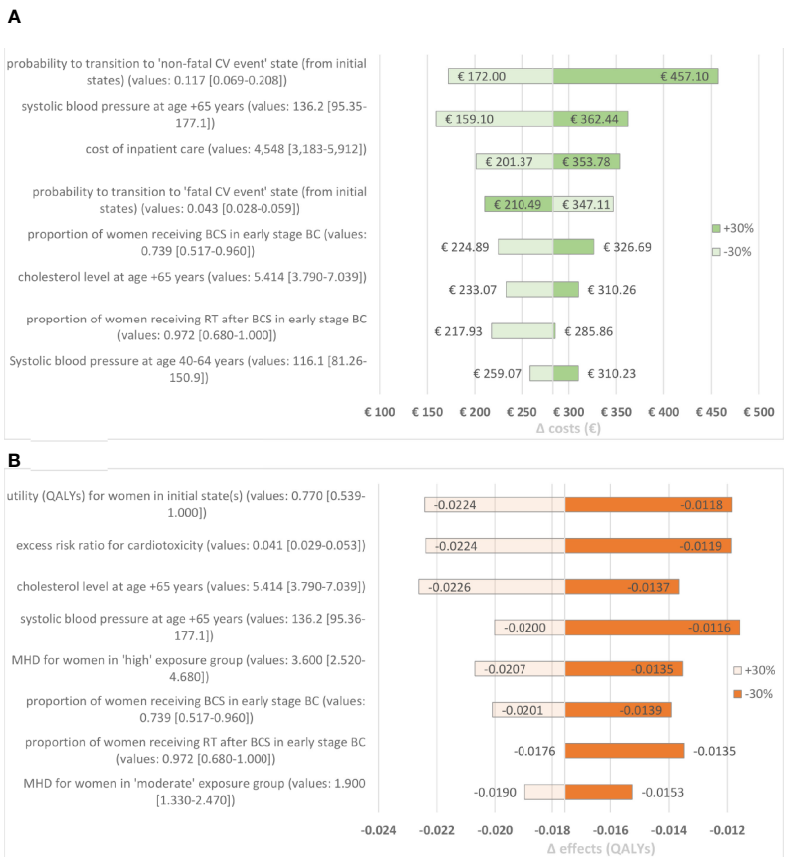

FIGURE 4 | **(A)** Tornado diagram for costs. This diagram represent the impact on incremental costs when setting the parameter to 70% and 130% of the base case value. The 8 most influential parameters are presented. **(B)** Tornado diagram for QALYs. This diagram represent the impact on incremental QALYs when setting the parameter to 70% and 130% of the base case value. The 8 most influential parameters are presented. BC, Breast Cancer; BCS, Breast Conserving Surgery; CV, Cardiovascular; MAS, Mastectomy; MHD, Mean Heart Dose; QALYs, Quality Adjusted Life Year.

radiotherapy is associated with four additional non-fatal and two additional fatal CV events in a cohort of 1,000 women over a time horizon of 20 years. In effect, the impact of taking radiation-induced cardiotoxic effects in account remains limited to an incremental cost of €275.10 per woman and incremental utility decrement of 0.017 per woman over 20 years.

Lundkvist et al. performed a CUA of proton beam therapy compared to conventional radiotherapy. As in our study, they choose non-fatal and fatal CV events as outcome measure in their Markov model. They expected 11.8 non-fatal CV events, and 3.3 fatal CV events per 100 55-year old women over a time horizon of 23 years (47). In our study, we observed, respectively, 9.6 events and 1.6 events in this age group over 20 years. Hence, the analyses produced a close match, supporting the assumptions in our model.

In our analysis, incremental costs increased with age, resulting in highest costs for older age groups. In effect, adjusting for age-specific incidence rates from different countries might reflect in higher costs for younger women. Direct costs were the main cost contributor in total costs. This is in line with results reported by Wilkins et al. who stated that direct health care costs contributed for over half of total CV-related costs in Europe (48). In our study the explanation lies in the increasing risk for non-fatal CV events at older age, accumulating in high inpatient care costs. The comparison between Lundkvist et al. and our results demonstrated earlier that the use of different data sources and extrapolating results over longer time horizons might reflect in -although small- differences between studies. As in our study, it remains a challenge to correctly extrapolate events over a long time horizon.

Our study is subject to several limitations. Firstly, it is important to notify that cardiotoxicity is not solely generated by radiation during BC treatment. Many studies in the field of cardio-oncology use the term 'treatment-induced cardiotoxicity' (49–51). Therefore, it could be considered a narrow approach to isolate radiotherapy from other cardiotoxic treatment factors (e.g. anthracyclines and HER-2 antagonists) (50, 52). On the other hand, in recent years the use of statins was widely introduced in order to reduce cardiovascular risk (53). Secondly, we assumed that a time horizon of 20 years would suffice in order to quantify radiation-induced cardiotoxicity since a reference paper in this field suggested an increased risk starting five years up until three decades after treatment (23). Hence, one might discuss a lifetime horizon in this matter is more appropriate. Especially for younger age groups in our model, the probability of an event after the analytic horizon of 20 years is plausible. In analogy to the EUROACTION study (54), we foresaw difficulties in modelling events in younger women because various covariables may intervene in longer time horizons. Especially in models concerning CV diseases, the probability of an event depends on several dependent parameters. Briggs et al. have suggested to introduce covariance in probabilistic analyses *via* Cholesky decomposition. Unfortunately, in most cases information is lacking regarding the underlying covariance structure to incorporate interdependency in the analyses. Therefore, it is common to treat all parameters as independent (55). We used numerous studies and data sources as vehicles for our economic evaluation. The possibility to combine data from different sources poses major opportunities for health economics, but this also increases the degree of uncertainty in the model (17). Thirdly, it is noteworthy that -though we strived to incorporate as much relevant evidence- some parameters were not included, possibly leading to an oversimplification of some aspects in the model. For example, the SCORE equations were updated in June 2021 allowing to add even more parameters to the prediction such as HDL-cholesterol and pre-existing diabetes mellitus (56). Also, the analyses of Darby et al. suggested that the risk for radiation-induced cardiotoxicity increased over time (23) whereas in our analyses the risk remains 4.1% per Gy. However, we employed the method which was proposed by Mulliez et al. to make estimations on the cardiovascular risk in breast cancer survivors (26). As argued by Briggs et al. highly complex model structures are not always more appropriate and simplified assumptions allow researchers to keep the model manageable (55). Finally, the authors would like to emphasize that results from health economic modelling need to be considered in the light of a specific context, meaning the cohort models contain various sources of evidence (e.g. transition probabilities, cost resources and health outcome measures) (17). Generalizability is only recommended to populations with a resembling context. Moreover, we intended to adopt a societal perspective, though we limited our cost resources to direct medical costs and indirect costs due to production loss. As with most CUA, it could be argued that we omitted some relevant cost resources (such as transportation costs, domestic help,…) or even costs in other sectors outside health care.

To our knowledge, our study is the first study which uses health economic modelling techniques to estimate the long-term costs and effects of radiation-induced cardiotoxicity. The use of these techniques can serve as a framework for the evaluation of the impact of a health risk on a population (57). Therefore, our results are applicable to future health economic research in the field of cardio-oncology. A noteworthy element in our analyses is the extent of MHD as influential parameter. Several studies revealed that heart doses were extensively higher in the past compared to current MHD (58, 59). Due to the awareness of long-term comorbidities in BC patients, substantial improvements on radiation therapy technology and protocols led to a reduction of the radiation dose to the heart (25). To date, research emphasizes the importance of continuing to invest in technological advances in heart-sparing techniques in order to further reduce the MHD (60). Our analyses showed that current radiation doses already evoke negligible incremental costs and disutility at population level. These findings emphasize the importance of previous technological innovations. Incremental costs and disutility must have been significantly higher in the past since MHD decreased substantially over the past decades. Hence, the cost of past investments in order to achieve current radiation dosages appears to be justified when considering the gains from cost and disutility reduction resulting from cardiotoxicity in BC survivors.

In conclusion, our analysis quantified more in detail the impact of radiation-induced cardiotoxicity in BC survivors. Our findings demonstrated that with current radiation techniques, minor overall mean incremental costs and disutility are to be expected over a time horizon of 20 years after primary BC treatment. Major research investments have been made in order to substantially decrease the MHD. Our findings highlight the importance of these investments when considered against the reduced costs and disutility from cardiovascular events as a result from these innovations. On the other hand, our analyses might also open a debate on potentially high opportunity costs of future investments since the expected marginal gains from further reducing the MHD may no longer outweigh the research budget.

DATA AVAILABILITY STATEMENT

All data referring to parameters is available in the article or supplementary material. Further inquiries should be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

EK: primary investigator and author (first draft preparation). AW: health economist and author (review and editing). MR: radiation oncologist (medical expertise) and senior author (review and editing). KP: health economist, principal investigator and senior author (review and editing). All the authors contributed to and agreed with the submitted manuscript.

FUNDING

This research was established within the Strategic Research Programme (zwaartepunt, SRP 53, 2019-2024) 'Societal Benefit of Markerless Stereotactic Body Radiotherapy: a Statistical Support based on Quantitative Imaging' (SMARTQI of the Vrije Universiteit Brussel).

ACKNOWLEDGMENTS

The authors would like to thank the Belgian Cancer Registry for supplying the requested aggregated dataset.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.869529/full#supplementary-material

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frontiers | Frontiers in Oncology

ORIGINAL RESEARCH
published: 21 April 2022
doi: 10.3389/fonc.2022.845037

Comparison of Deep Inspiration Breath Hold Versus Free Breathing in Radiotherapy for Left Sided Breast Cancer

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OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Radiation Oncology, a section of the journal Frontiers in Oncology

Received: 29 December 2021
Accepted: 29 March 2022
Published: 21 April 2022

Citation:
Lu Y, Yang D, Zhang X, Teng Y, Yuan W, Zhang Y, He R, Tang F, Pang J, Han B, Chen R and Li Y (2022) Comparison of Deep Inspiration Breath Hold Versus Free Breathing in Radiotherapy for Left Sided Breast Cancer. Front. Oncol. 12:845037. doi: 10.3389/fonc.2022.845037

Objectives: Modern breast cancer techniques, such as the deep inspiration breath-hold (DIBH) technique has been applied for left-sided breast cancer. Whether the DIBH regimen is the optimal solution for left-sided breast cancer remains unclear. This meta-analysis aims to elucidate the differences of DIBH and free-breathing (FB) for patients receiving radiotherapy for left-sided breast cancer and provide a practical reference for clinical practice.

Methods: Relevant research available on PubMed, Embase, Cochrane Library, and the Web of Science published before November 30, 2021 was independently and systematically examined by two investigators. Data were extracted from eligible studies for assessing their qualities and calculating the standardized mean difference (SMD) and 95% confidence intervals (CIs) using Review Manager software 5.4 (RevMan 5.4).

Results: Forty-one studies with a total of 3599 left-sided breast cancer patients were included in the meta-analysis. Compared with FB, DIBH reduced heart dose (D_{mean}, D_{max}, V30, V10, V5), left anterior descending branch (LAD) dose (D_{mean}, D_{max}), ipsilateral lung dose (D_{mean}, V20, V10, V5), and heart volume significantly. Lung volume increased greatly, and a statistically significant difference. For contralateral breast mean dose, DIBH has no obvious advantage over FB. The funnel plot suggested this study has no significant publication bias.

Conclusions: Although DIBH has no obvious advantage over FB in contralateral breast mean dose, it can significantly reduce heart dose, LAD dose, ipsilateral lung dose, and heart volume. Conversely, it can remarkably increase the ipsilateral lung volume. This study suggests that soon DIBH could be more widely utilized in clinical practice because of its excellent dosimetric performance.

Keywords: left sided breast cancer, radiotherapy, free breathing, deep inspiration breath hold, meta-analysis

INTRODUCTION

Breast cancer is a significant global public health problem and the leading cause of cancer mortality in women (1). Adjuvant radiation therapy has a major role managing this disease, reducing the risk of local recurrence and breast cancer-specific mortality (2). It is certain that radiotherapy is an effective way to treat breast cancer, and significantly prolongs the survival time. However, breast cancer radiation therapy is also associated with higher cardiac and pulmonary toxicity [e.g., radiation-related heart disease (RRHD) (3) and radiation pneumonia (RP) (4)] with an increased risk of secondary cancer (3, 5–9). Darby et al. showed the risk of major coronary events induced by radiation increased linearly with the mean heart dose (MHD) by 7.4% per gray, with no threshold dose (3). Clarke et al. compared a group of irradiated patients with non-irradiated patients and found a significant increase in mortality rate, mainly for heart disease and lung cancer with a rate ratio of 1.27 and 1.78, respectively (2).

Therefore, with patients receiving radiotherapy for breast cancer substantial efforts have been made to develop techniques that reduce heart and lung dose, such as Deep inspiration breath-hold (DIBH). This simple technique reduces cardiac exposure by lung expansion which physically displaces the heart out of the treatment field. There are several approaches for performing DIBH, in particular active breath control, external infrared box marker, and optical surface monitor (10). Studies have demonstrated that DIBH, for left-sided breast cancer patients, can reduce the cardiac dose compared with free-breathing (FB) (5, 9, 11–13). It is noteworthy that the technique has high repeatability and stability in the whole treatment process (14).

Although many studies show DIBH technology is correlated to heart dose, LAD dose, ipsilateral lung dose, contralateral breast dose, heart volume, and ipsilateral lung volume, we have reached an understanding that DIBH is critical and superior to free-breathing (FB) in radiotherapy for left-sided breast cancer. However, there are many small sample studies, which gives a lack confidence. Therefore, we searched all of the controlled studies of DIBH and FB in radiotherapy of the left breast and conducted this meta-analysis. It is noteworthy that the research groups with different radiotherapy techniques (3D-CRT, IMRT, or VMAT), postures (supine or prone position), and prescribed dose schemes (CF or HF) in the same study were included in this meta-analysis.

METHODS

Search Strategy

Using a combination of medical subject heading (MeSH) terms and/or free text words such as, "breast cancer", "radiotherapy" and "deep inspiration breath-hold or DIBH", we thoroughly searched four medical databases including PubMed, Embase, Cochrane library, and Web of Science for relevant studies published before November 30, 2021. There was no limitation on the language of published studies. Furthermore, references of selected studies were manually reviewed, and literature searching and screening were independently performed by two investigators. Disagreement was resolved through discussion with a third investigator.

Inclusion Criteria

All studies included were following the principles of PICOS (Participants, Intervention, Comparison and Outcomes, Study design). Inclusion criteria were as follows: (1) Participants [P]: Patients were pathologically diagnosed with left-sided breast cancer without distant metastasis. (2) Intervention [I]: Patients in the experimental group received a DIBH regimen. (3) Comparison [C]: Free-breathing (FB) regimen was the intervention in the control group. (4) Outcomes [O]: The outcomes included dosimetric indicators of heart, left anterior descending artery, ipsilateral lung, and contralateral breast: the mean dose (D_{mean}), the maximum dose (D_{max}), and the percentage of the organ volume receiving at least 5 Gy (V5), 10 Gy (V10), 20 Gy (V20), 25 Gy (V25) and 30 Gy (V30). (5) Study design [S]: randomized controlled trials (RCTs) and observational studies, including cohort and case-control studies. It should be noted that trials with different fractionation regimens and prescribed doses were included in this study.

Exclusion Criteria

Articles satisfying any of the following items were excluded: (1) Reviews, case reports, letters, and abstracts; (2) Low research quality or having a high risk of bias; (3) Lacking available data that could be pooled.

Data Extraction

The following information was independently extracted from the included studies by two researchers (Mr. Yang and Mr. Teng): First author, year of publication, country, study design, age, DIBH type, clinical tumor stage, sample size, detailed treatment plan, and outcomes of the various subgroups. Dispute regarding data extraction was arbitrated by a third investigator (Mr. Tang).

Quality Assessment

To assess the risk of bias in nonrandomized studies Newcastle-Ottawa Scale (NOS) (15) was introduced, involving three perspectives: Selection, comparability, and outcome of the studies. Using a 0-9 scale, 4 points were graded for selection, 2 for comparability, and 3 for outcomes. Studies with 6 points or higher were considered high quality (16).

Statistical Analysis

The pooled statistics were performed using RevMan software version 5.4 (Cochrane Collaboration, Oxford, UK). Standardized mean difference (SMD) and 95% CI were selected as the effect indicator to analyze measurement data. Heterogeneity was evaluated between trials through the Cochrane Q test and the I^2 statistic, which quantified the proportion of total variation caused by heterogeneity instead of chance (17). If the P-value of the Q test was >0.10 and I^2< 50%, a fixed-effects model was used for data with non-significant heterogeneity. Otherwise, a random-effects

model was used for data with significant heterogeneity (18, 19). Furthermore, the sensitivity analysis was also applied to examine the potential influence of an individual study on the overall assessment, which involved removing one study each time and pooling the remaining trials. A funnel plot was used to understand the bias of the literature publication. If the points in the funnel plot are symmetrically distributed on both sides of the middle dashed line and concentrate in the center, the possibility of publication bias is low. If not, the possibility of publication bias could be high.

RESULTS

Study Selection

Initially, after excluding 236 duplicates, 232 articles were retrieved through preliminary searches in PubMed, Embase, the Cochrane Library, and Web of Science. Then, 62 unqualified articles were eliminated through reviewing titles and abstracts. After a full-text reading, 41 qualified articles were assessed for design and quality (5, 7, 20, 13, 21–57). The detailed process of the study selection is shown in **Figure 1**.

Study Characteristics

Finally, 41 studies (5, 7, 13, 20–57) totaling 3599 left-sided breast cancer patients were included in our meta-analysis. All articles included were retrospective studies and identified as high quality by the Newcastle–Ottawa Scale (15). **Table 1** summarizes the baselines information of the 41 included studies. Each group of data shall be counted independently when multiple groups of data are in the same study.

Heart Dose

Heart dose data (D_{mean}, D_{max}, V30, V10, and V5) were extracted from 38 articles which studied 3507 patients. The random-effects model was applied due to the significant between-study heterogeneity ($I^2 \geq 50\%$, $P \leq 0.10$). The pooled results showed there was a difference between the DIBH group and FB group. By combining the results with clinical information from the

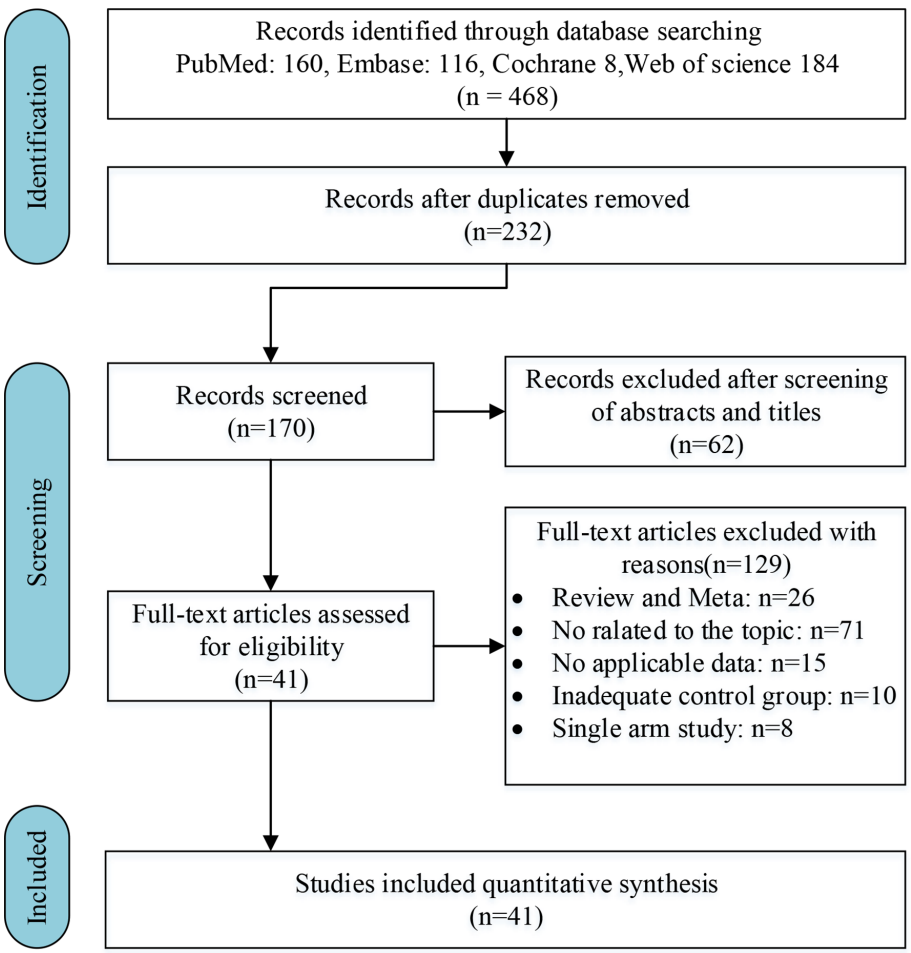

FIGURE 1 | Flow chart of the search process for the meta-analysis.

TABLE 1 | Characteristics of the studies included in the meta-analysis.

First author (year of publication)	Total Patients (DIBH/FB)	Clinical stage	Median age (years)	Prescription dose(Gy)/Fractions(F)	DIBH types	Study type	NOS score
Angela 2017 (20)	64 (32/32)	NA	NA	50 Gy/25 F	RPM	Retrospective	6
Bruzzaniti 2013 (CF) (21)	16 (8/8)	NA	51	50 Gy/25 F	RPM	Retrospective	7
Bruzzaniti 2013 (HF) (21)	16 (8/8)	NA	51	34 Gy/10 F	RPM	Retrospective	7
Chatterjee 2018 (22)	70 (50/20)	NA	NA	40 Gy/15 F	RPM	Retrospective	6
Chi. F. 2015 (23)	62 (31/31)	I or II	39.5	50 Gy/25 F	ABC	Retrospective	8
Christina 2021 (24)	194 (97/97)	NA	54	40.05-50.4 Gy/15 -28 F	RPM	Retrospective	7
Comsa 2014 (25)	60 (30/30)	NA	<50	50 Gy/25 f	ABC	Retrospective	6
Dincoglan 2013 (26)	54 (27/27)	NA	<65	50 Gy/25 f	ABC	Retrospective	7
Dolezel 2021 (27)	200 (100/100)	cT1-3N0-2	59	48.6 Gy/27 f	NA	Retrospective	7
Eldredge 2015 (28)	172 (86/86)	T1–3N0–3M0	52	50 Gy/25 f	ABC	Retrospective	9
Ferini 2021 (29)	232 (116/116)	I-II	56	40.5-50 Gy/15-25 f	RPM	Retrospective	8
Goyal 2020 (30) (prone position)	28 (14/14)	NA	>18	40-42.6 Gy/15-16 f	RPM	Retrospective	7
Hammadi 2018 (31)	108 (54/54)	NA	41	50 Gy/25 f	NA	Retrospective	6
Hepp 2015 (32)	40 (20/20)	pTis–pT1 pN0	NA	50 Gy/25 f	Catalyst	Retrospective	7
Jensen 2017 (33)	44 (22/22)	pT1-2N0M0, ductal carcinoma	58	50 Gy/25 f	laser-based DIBH system	Retrospective	7
Jiheon 2020 (34)	150 (75/75)	Invasive breast cancer or ductal carcinoma	NA	40-42.5 Gy/15-16 f	Medspira Breath-Hold	Retrospective	7
Kunheri 2017 (35)	90 (45/45)	I–IIIA	45.2	40 Gy/15 f	ABC	Retrospective	8
Lastrucci 2017 (36)	46 (23/23)	NA	NA	50 Gy/25 f	Medspira Breath-Hold	Retrospective	7
Lawler 2017 (37)	56 (28/28)	NA	57.39	40.05–50 Gy/15–25 f	RPM	Retrospective	7
Lee 2013 (38)	50 (25/25)	≤T2 and ≤N1a	29	50.4 Gy/28f	Abches	Retrospective	8
Lin 2019 (39)	184 (63/121)	Tis, I, or II	51.53	50 Gy/25 f	ABC	Retrospective	8
Liuwei 2021 (40)	22 (11/11)	NA	NA	42.4 Gy/16f	NA	Retrospective	6
Misra 2021 (41)	60 (30/30)	I-III	50	40 Gy/15f	RPM	Retrospective	9
Mohamad 2017 (42)	44 (22/22)	NA	NA	50 Gy/25 f	ABC	Retrospective	6
Nissen 2013 (43)	227 (144/83)	NA	55.5 (DIBH) 64 (FB)	50 Gy/25 f	ABC	Retrospective	9
Pham 2016 (44) (IMRT Group)	30 (15/15)	NA	NA	50 Gy/25 f	RPM	Retrospective	6
First author (year of publication)	Total Patients (DIBH/FB)	Clinical stage	Median age (years)	Prescription dose(Gy)/Fractions(F)	DIBH types	Study type	NOS score
Pham 2016 (44) (VMAT Group)	30 (15/15)	NA	NA	50 Gy/25 f	RPM	Retrospective	6
Rochet 2015 (45)	70 (35/35)	Tis-T3N+M0	51	42.4–50-50.4 Gy/16–25-28 f	AlignRT	Retrospective	7
Saini 2018 (46)	66 (33/33)	T1-2N0	NA	42.56 Gy/16 f	DIBH (other)	Retrospective	7
Saini 2019 (7) (prone position)	50 (25/25)	T1-2N0	NA	42.56 Gy/16 f	DIBH (other)	Retrospective	7
Saini 2019 (7) (supine position)	50 (25/25)	T1-2N0	NA	42.56 Gy/16 f	DIBH (other)	Retrospective	7
Sakka 2017 (47) (IMRT Group)	40 (20/20)	NA	<70	50.4 Gy/28 f	RPM	Retrospective	7
Sakka 2017 (47) (VMAT Group)	40 (20/20)	NA	<70	50.4 Gy/28 f	RPM	Retrospective	7
Sakyanun 2020 (48)	50 (25/25)	NA	NA	50 Gy/25 f	RPM	Retrospective	6

(Continued)

TABLE 1 | Continued

First author (year of publication)	Total Patients (DIBH/FB)	Clinical stage	Median age (years)	Prescription dose(Gy)/Fractions(F)	DIBH types	Study type	NOS score
Schönecker 2016 (49)	18 (9/9)	NA	46.9	50 Gy/25 f	Catalyst/Sentinel	Retrospective	7
Shim 2012 (50)	20 (10/10)	T1N0, T2N0,T2N1	44	50 Gy/25 f	NA	Retrospective	6
Simonetto 2019 (51)	198 (89/89)	Tis-T4	57	40-50 Gy/15-25 f	Catalyst/Sentinel	Retrospective	9
Stranzl 2009 (52)	22 (11/11)	NA	51	NA	RPM	Retrospective	6
Sunmin 2021 (53)	30 (15/15)	T1-2N0	54	50 Gy/25 f	RPM	Retrospective	9
Tanguturi 2015 (54)	148 (110/38)	All stages	58/49.5	50 Gy/25 f	AlignRT	Retrospective	8
Vikström 2011 (55)	34 (17/17)	NA	60	50 Gy/25 f	RPM	Retrospective	6
Wang 2012 (13)	106 (53/53)	NA	52	42.4–50 Gy/16–25 f	ABC	Retrospective	8
Wiant 2015 (56)	50 (25/25)	NA	NA	50.4 Gy/28 f	Philips Bellows system	Retrospective	7
Yamauchi 2020 (5)	170 (85/85)	NA	49.3	50 Gy/25 f	RPM	Retrospective	7
Zhao-Feng 2018 (57) (3D-CRT Group)	44 (22/22)	NA	48	50 Gy/25 f	RPM	Retrospective	7
Zhao-Feng 2018 (57) (IMRT Group)	44 (22/22)	NA	48	50 Gy/25 f	RPM	Retrospective	7

DIBH, deep inspiration breath hold; FB, free breathing; NOS, Newcastle–Ottawa Scale; CF, conventional fractionation; HF, Hypofractionation; ABC, active breathing coordinator; RPM, real-time position management; AlignRT, a realtime surfacetracking system; VMAT, volumetric modulated arc therapy; IMRT, intensity-modulated radiation therapy; 3D-CRT, 3-dimensional conformal radiotherapy; NA, not available.

included studies, it was indicated that DIBH technology can decrease heart doses more effectively than the FB group. The results are presented in **Figures 2** and **3**, D_{mean} (SMD = -1.28, 95% CI: -1.42 - 1.13, P<0.01), D_{max} (SMD = -1.86, 95% CI: -2.26 ~ -1.46, P<0.01), V30 (SMD = -1.23, 95% CI: -1.49 ~ 0.97 P<0.01), V10 (SMD = - 1.40, 95% CI: -1.65 ~ -1.15, P<0.01), V5 (SMD = -1.58, 95% CI: -2.05 ~ -1.12, P<0.01).

LAD Dose

Twenty-seven studies involving 2146 patients were eligible for analyzing the LAD dose (D_{mean} and D_{max}). Significant heterogeneity was identified (I^2≥50%, P ≤ 0.10) and as a result, a random-effects model was employed to calculate the pooled data. The data demonstrated that the LAD dose (D_{mean} and D_{max}) of the DIBH group was significantly lower than that of the FB group (D_{mean}: SMD = -1.35, 95% CI: -1.57 ~ -1.13, P<0.01; D_{max}: SMD = -1.26, 95% CI: -1.61 ~ -0.90, P<0.01) (**Figure 4**).

Ipsilateral Lung Dose

Ipsilateral lung dosimetric indicators (D_{mean}, V20, V10, and V5) were extracted from 33 studies with 2768 patients. The heterogeneity test showed statistically significant differences among the studies (I^2 ≥ 50%, P ≤ 0.10), and therefore, a random-effects model was introduced. Compared to the FB group, left-sided breast cancer patients could benefit more from DIBH technology. The results are presented in **Figures 5** and **6**, D_{mean} (SMD = - 0.55, 95% CI: -0.73 ~ -0.37, P<0.01), V20 (SMD = -2.62, 95% CI: -3.37 ~ -1.87 P<0.01), V10 (SMD = -2.71, 95% CI: -3.71 ~ -1.72, P<0.01), V5 (SMD = - 2.08, 95% CI: -3.11 ~ -1.04, P<0.01).

Contralateral Breast Mean Dose

Eight studies, with 578 left-sided breast cancer patients in total, were included in this analysis. During the analysis, we found no significant between-study heterogeneity (I^2 = 0%; p = 0.53), and a fixed-effects model was used. The combined analysis showed that there was no significant difference in contralateral breast mean dose between the two groups and there was no statistical significance (SMD = -0.19, 95% CI: -0.36 ~ -0.03, P=0.02) (**Figure 7**).

Heart Volume

Heart volume was reported in eleven studies with a total of 832 patients. The fixed-effects model was applied due to no significant between-study heterogeneity (I^2 = 32%; p = 0.14). In comparison with the FB group, the application of DIBH technology makes cardiac volume compression in patients with left-sided breast cancer. (SMD = -0.32, 95% CI: -0.46 ~ -0.18, P<0.01) (**Figure 8**).

Ipsilateral Lung Volume

Fifteen studies involving 1599 left-sided breast cancer patients were eligible for analysis. The fixed-effects model was conducted for no significant between-study heterogeneity (I^2 = 0%; p = 0.55). Meta-analysis showed that DIBH technology significantly increased the ipsilateral lung volume (SMD = 2.35, 95% CI: 2.22 ~ -2.48, P<0.01) (**Figure 9**).

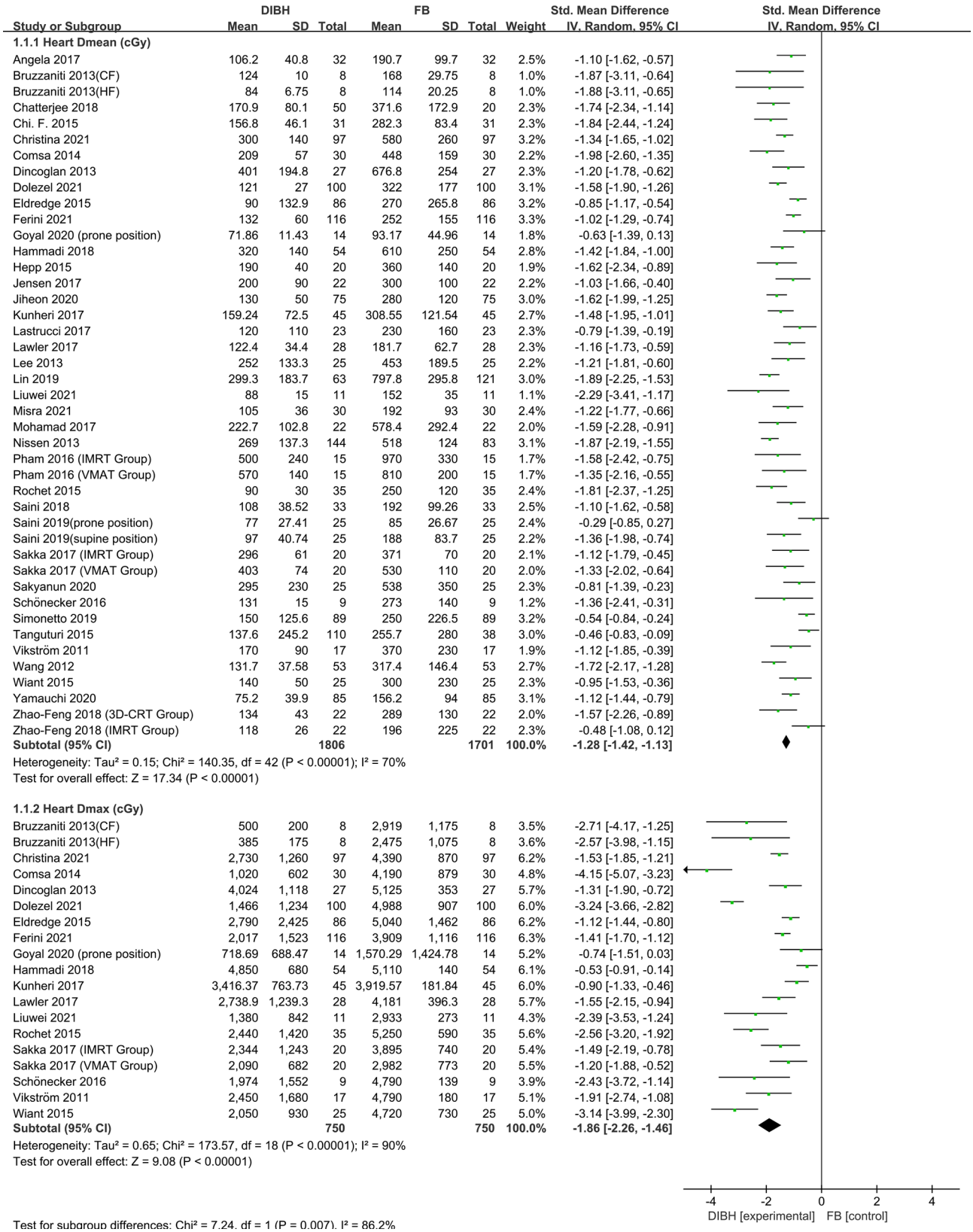

FIGURE 2 | Forest plot of heart dose (D_{mean} and Dmax) between the DIBH group and FB group.

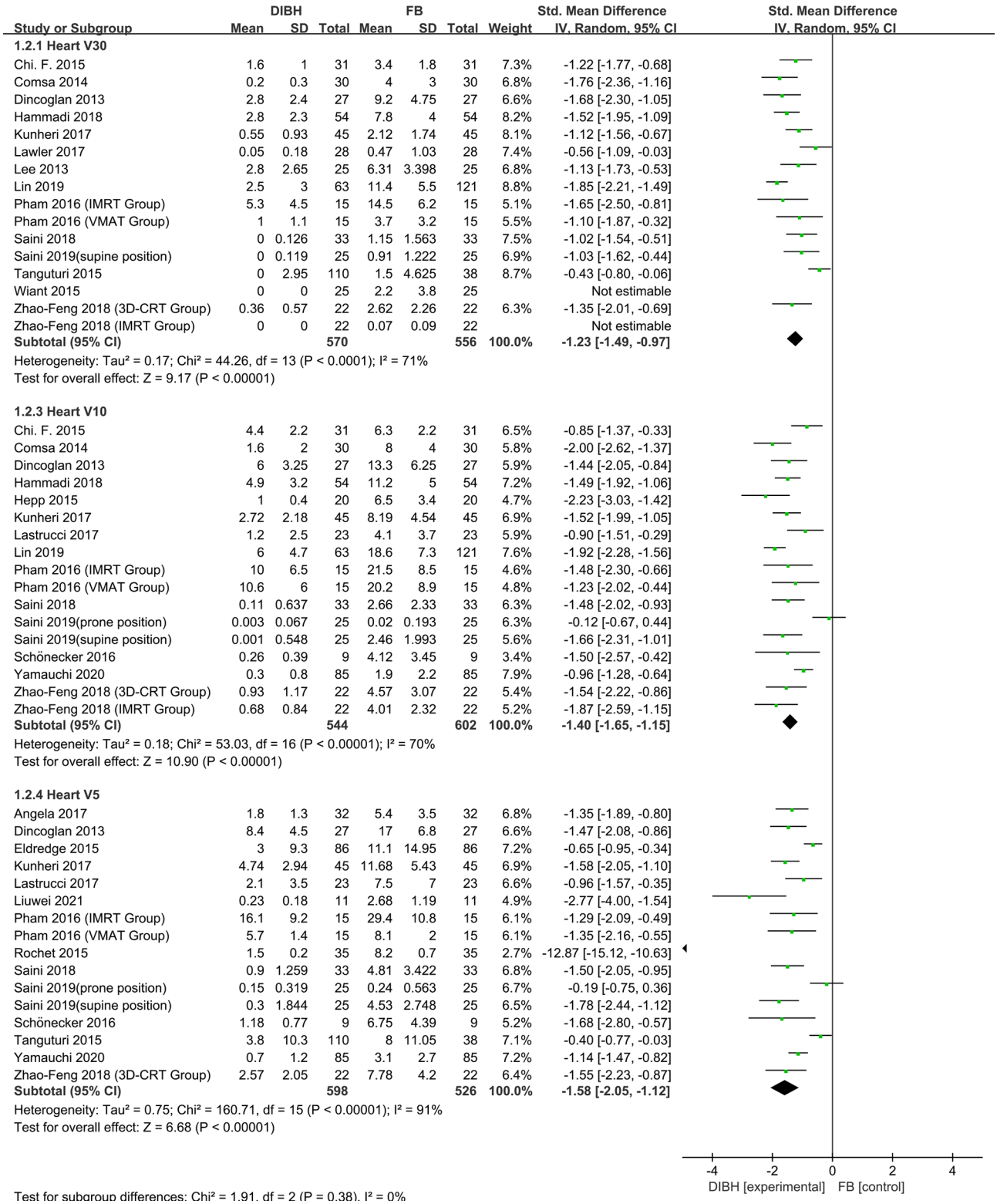

FIGURE 3 | Forest plot of heart dose (V30, V10 and V5) between the DIBH group and FB group.

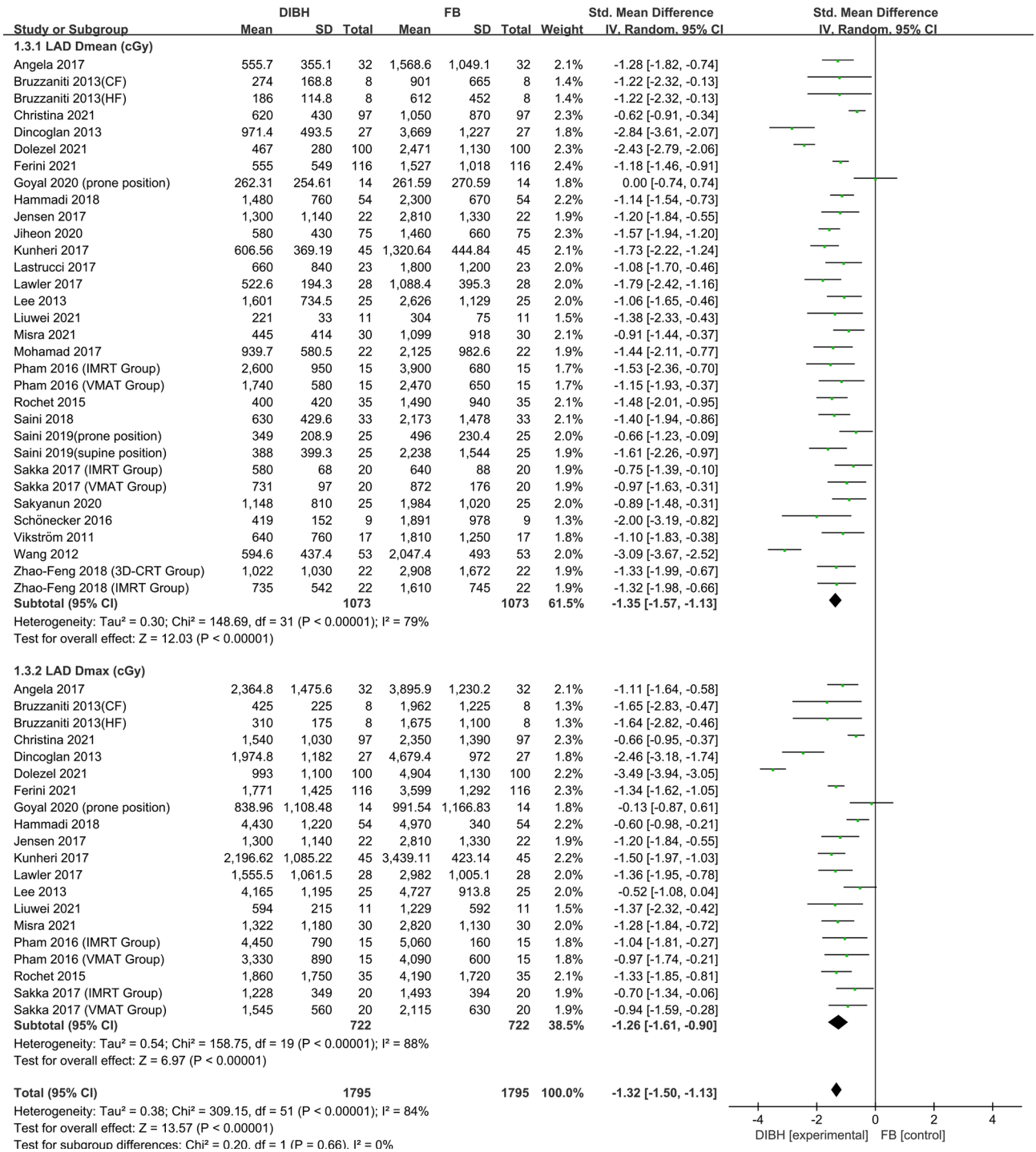

FIGURE 4 | Forest plot of LAD dose (D_{mean} and *D*max) between the DIBH group and FB group.

Publication Bias

A funnel plot was applied for the assessment of publication bias in the literature, tests for the funnel plot asymmetry were applied if there were at least 10 studies included in the meta-analysis. From the funnel plot of different indicators (**Figure 10**), it is evident that the point estimates are symmetrically distributed on both sides, centralized in the middle, therefore showing no evidence of publication bias.

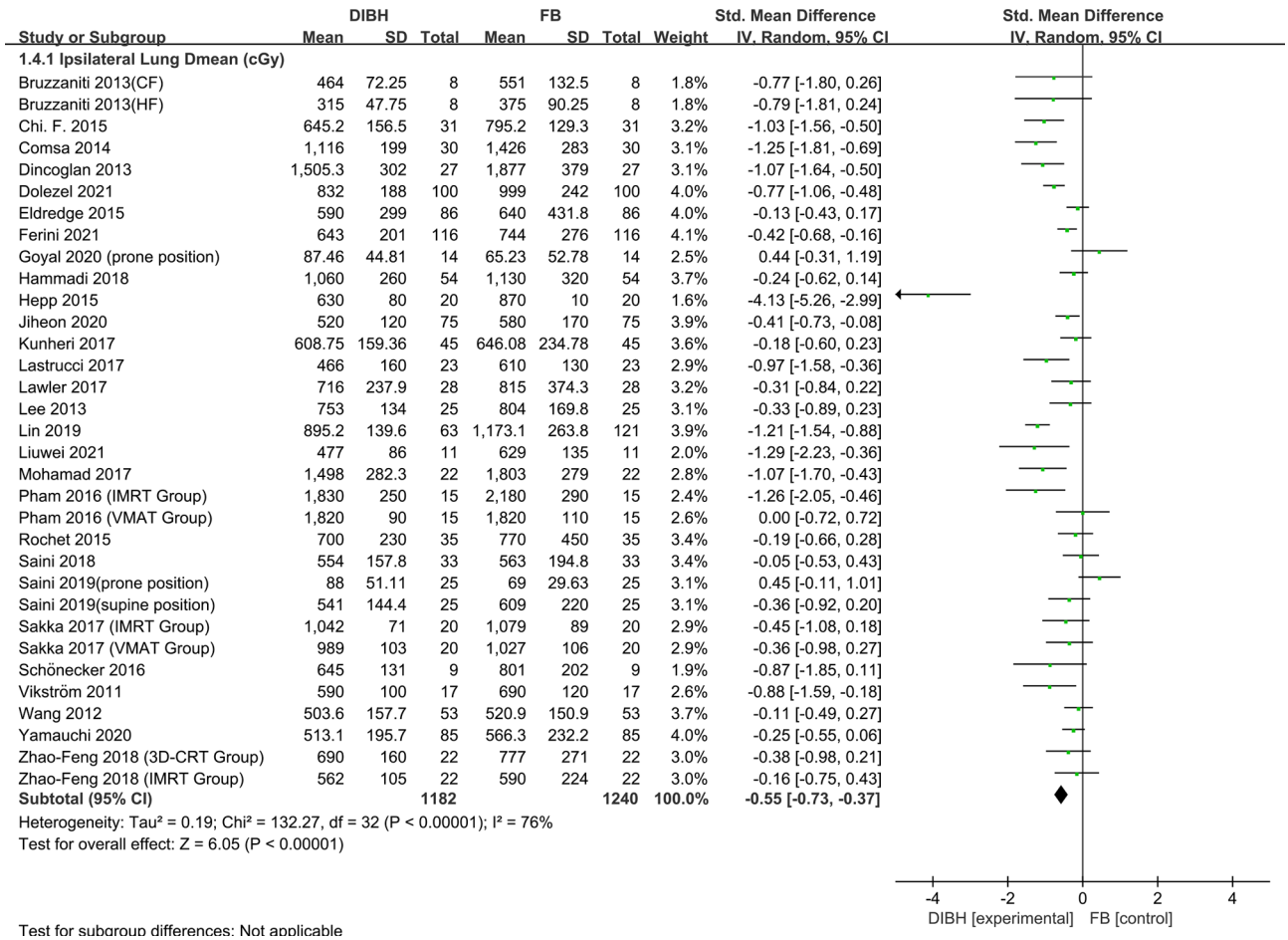

FIGURE 5 | Forest plot of ipsilateral lung dose (D_{mean}) between the DIBH group and FB group.

DISCUSSION

There are many studies on the incidence of RRHD caused by radiotherapy for breast cancer. The research of Darby et al. (3) in 2013 showed that exposure of the heart to ionizing radiation during radiotherapy for breast cancer increases the subsequent rate of ischemic heart disease. The increase is proportional to the mean dose to the heart, beginning within a few years after exposure, and continues for at least 20 years. Women with preexisting cardiac risk factors have greater absolute increases in risk from radiotherapy than other women. Additionally, further studies indicate that LAD coronary artery doses may be particularly relevant to RRHD risks, since this artery is a common site of atherosclerosis inducing myocardial infarction. It is the site of high doses in many left-breast cancer radiotherapy regimens, and may well contribute to radiation-induced heart disease (58). Some recent research has focused on the relationship between the average cardiac dose and the incidence of adverse events. One such research conducted by Van den Bogaard et al. concluded that the cumulative incidence of acute coronary events increased by 16.5% per Gy (59). A study by Dutch et al. showed that the risk of myocardial infarction increased linearly as the mean of the whole heart dose increased, with an excess risk ratio of 6.4% per Gy (60).. In another Ebbe Laugaard Lorenzen et al. study, it was demonstrated that for female patients receiving tangential field irradiation, the linear increase in the excess odds ratio of major coronary events per gray of mean heart dose was 19% (61). Therefore, to reduce the incidence of RRHD, the deposition dose of heart and LAD should be low enough. In this paper, we respectively studied the dosimetric indexes of heart and LAD. The results implied that the dose of the heart and LAD in the DIBH group was significantly lower than that in the FB group. The meta-analysis results of all subgroups of cardiac dose (D_{mean}, D_{max}, V30, V10, and V5) and LAD dose subgroup (D_{mean}, D_{max}) support this conclusion unanimously (**Figures 2–4**). We have reason to believe that DIBH may reduce RRHD more effectively by reducing the dose to the heart and LAD, such as ischemic heart disease, acute coronary event and myocardial infarction. Moreover, the results of this study infer that different radiotherapy techniques (3D-CRT, IMRT or VMAT), postural design (supine or prone position) and prescribed dose schemes

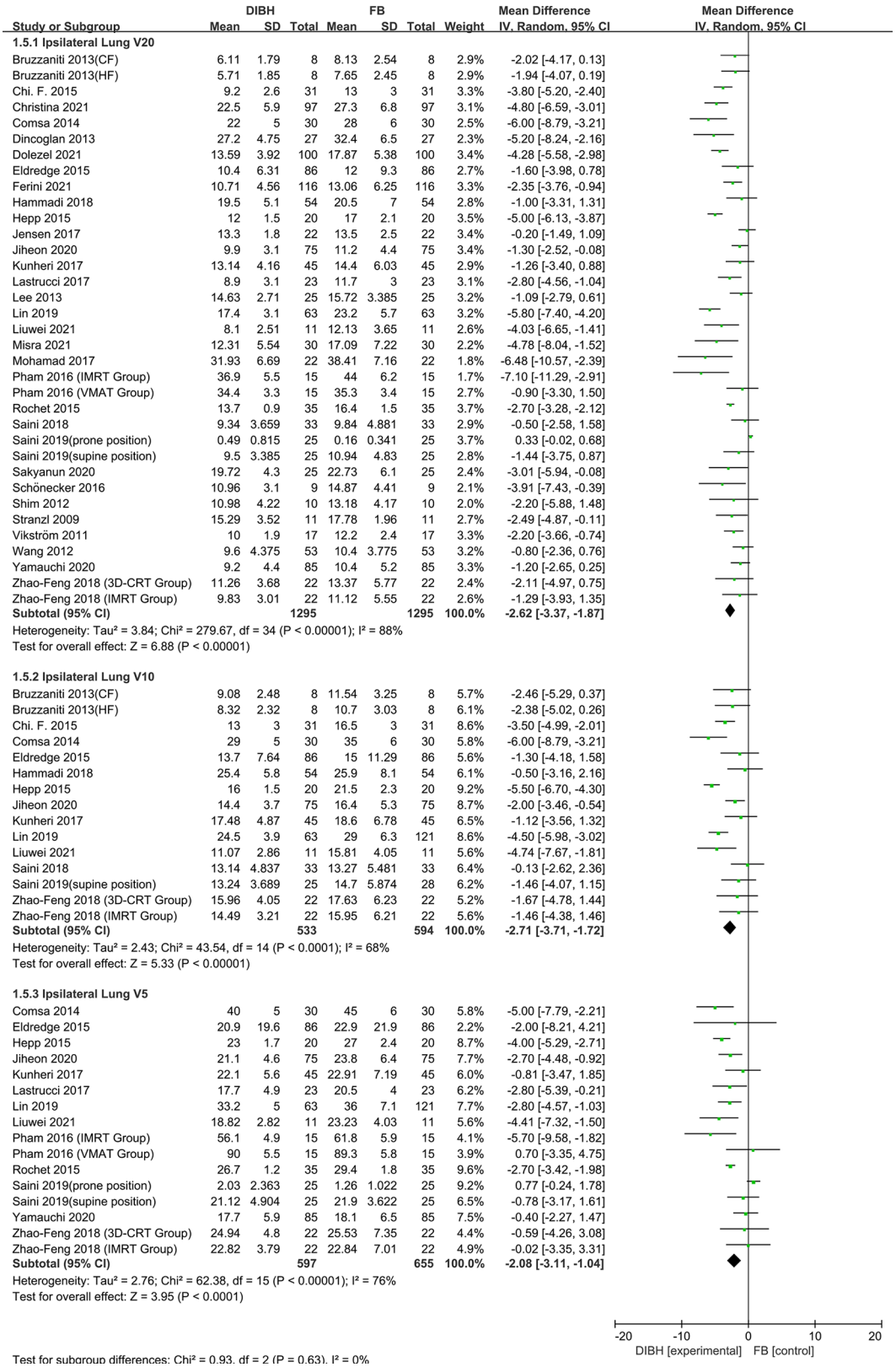

FIGURE 6 | Forest plot of ipsilateral lung dose (V20, V10 and V5) between the DIBH group and FB group.

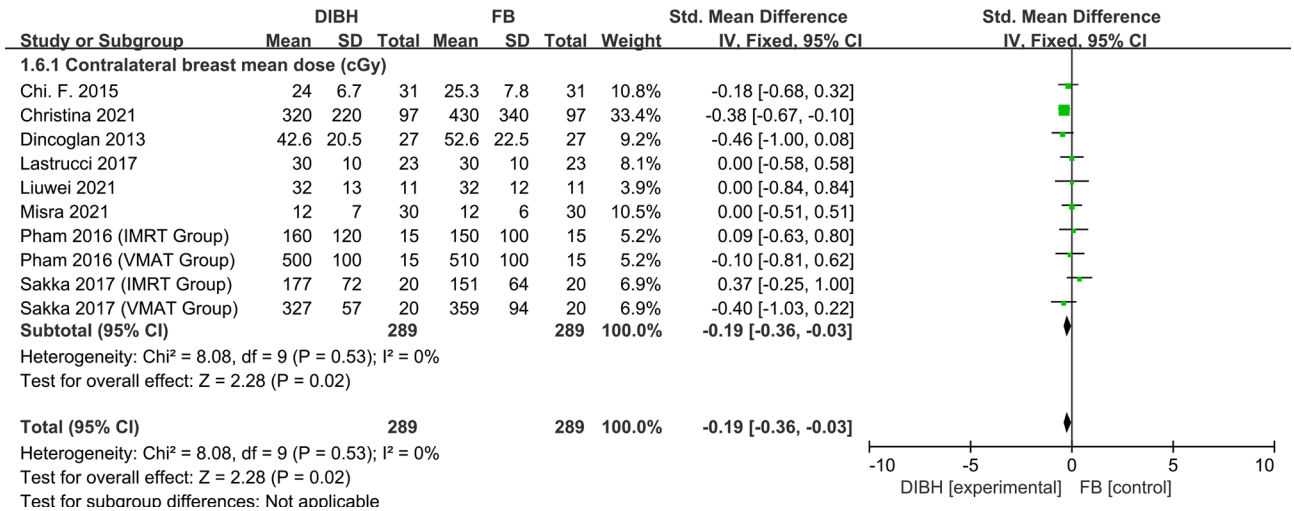

FIGURE 7 | Forest plot of contralateral breast mean dose between the DIBH group and FB group.

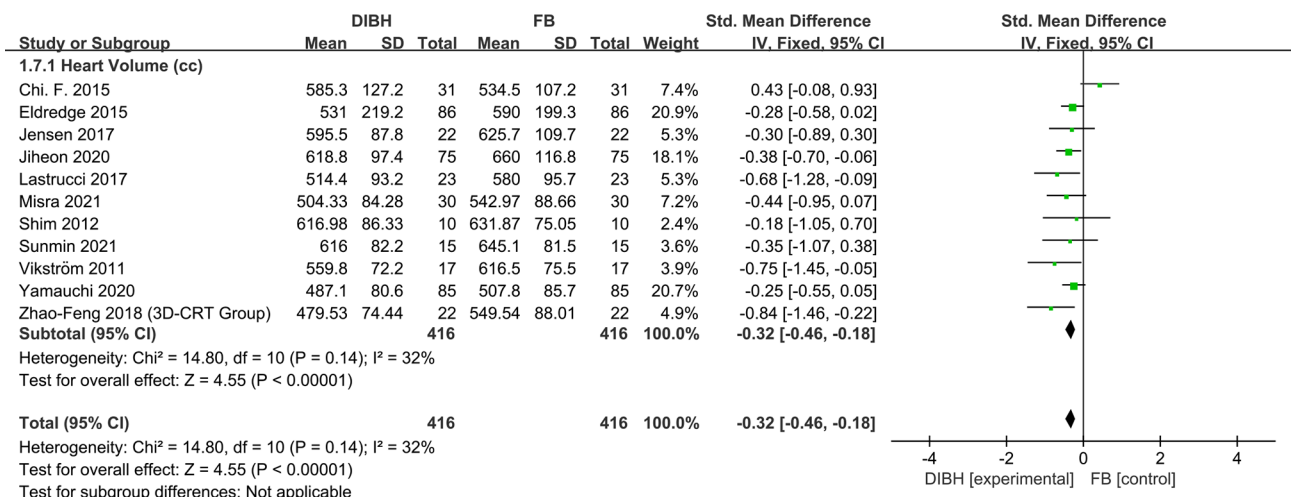

FIGURE 8 | Forest plot of heart volume between the DIBH group and FB group.

(CF or HF) did not affect the dose reduction advantages of DIBH compared with FB in the heart and LAD.

In 1998, Kwa et al. (62) conducted a large multicenter study of 530 people on the relationship between the incidence of radiation pneumonitis and dose distribution in the lungs. Their results showed that increasing pneumonitis rate was observed with increasing mean lung dose in all centers. Especially in the low dose range of 4 to 16 Gy, the incidence rate of pneumonia in the breast group was 1.4%. Therefore, the mean lung dose can be used as a useful predictor of the risk of radiation pneumonia. Additionally, research conducted by Gokula et al. and Kasmann et al. implied that Locoregional radiotherapy increased the mean lung dose, and ipsilateral lung volume receiving 20 Gy (V20) >30% have been identified as risk factors for RP (63, 64). In this study, ipsilateral lung dosimetric indicators (D_{mean}, V20, V10, and V5) were extracted from 33 studies totaling 2768 patients. Compared to the FB group, left-sided breast cancer patients could benefit more from DIBH technology. The subgroup analysis results are presented in **Figures 5** and **6**, D_{mean} (SMD = -0.55, 95%, CI: -0.73 ~ -0.37, P<0.01), V20 (SMD = -2.62, 95% CI: -3.37 ~ -1.87 P < 0.01), V10 (SMD = -2.71, 95% CI: -3.71 ~ -1.72, P <0.01), V5 (SMD = -2.08, 95% CI: -3.11 ~ -1.04, P<0.01). We can conclude that DIBH technology may reduce the incidence of RP by reducing the mean lung dose, V20, V10, and V5. However, there are a few exceptions. It can be seen from the forest plot (**Figures 5** and **6**) that DIBH did not perform better than FB in all prone position groups. Therefore, large sample size experiments are needed to focus on the difference

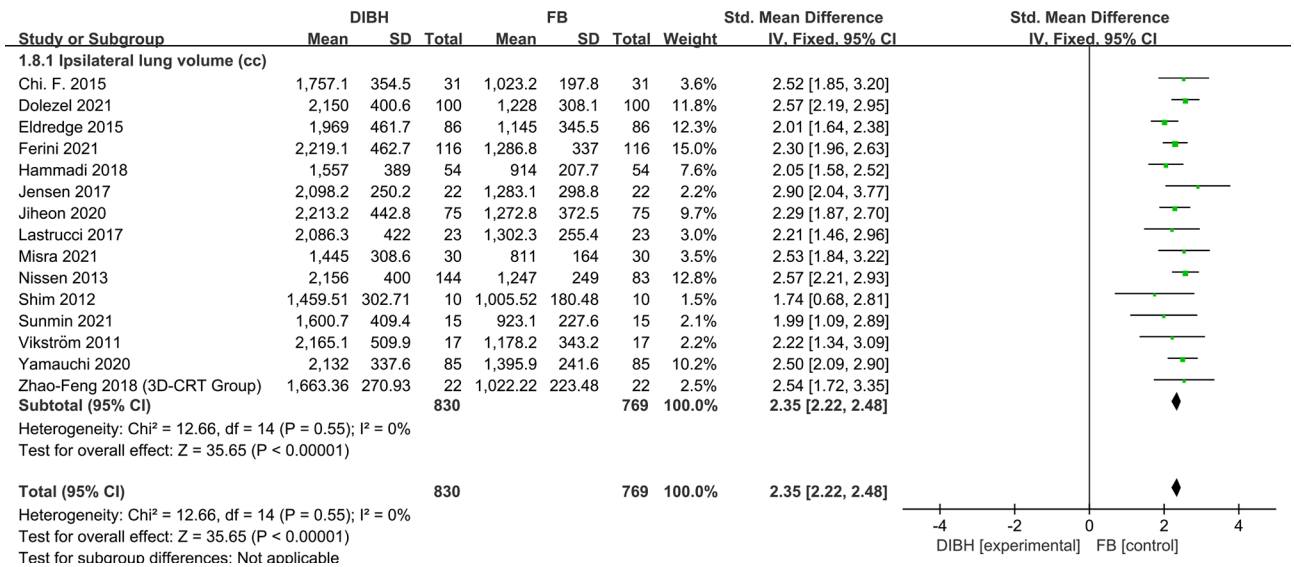

FIGURE 9 | Forest plot of ipsilateral lung volume between the DIBH group and FB group.

between DIBH technology and FB in dissimilar postures. In addition, the results of this study infer that different radiotherapy techniques (3D-CRT, IMRT or VMAT) and prescribed dose schemes (CF or HF) did not affect the dose reduction advantages of DIBH compared with FB in the ipsilateral lung.

Further, we counted and analyzed the mean dose of contralateral breast, heart volume, and ipsilateral lung volume. The combined analysis showed between the two groups there was no significant difference in contralateral breast mean dose and there was no statistical significance (SMD = - 0.19, 95% CI: -0.36 ~ -0.03, P=0.02). Meanwhile, results indicated that the ipsilateral lung volume increased significantly in the DIBH group (SMD = 2.35, 95% CI: 2.22 ~ 2.48, P<0.01), while the heart volume was compressed (SMD = -0.32, 95% CI: -0.46 ~ -0.18, P<0.01). This phenomenon is not difficult to understand, because DIBH is a simple technique used to reduce cardiac exposure by lung expansion which physically displaces the heart out of the radiation field. Objectively speaking, the use of DIBH technology expands the lung volume, which in turn makes the contralateral breast farther away from the radiation field, and finally the contralateral breast should have a lower mean dose. However, in the FB group, the contralateral breast was also almost outside the field, which made the DIBH group have no significant advantage in reducing the mean breast dose compared with the FB group.

Potential limitations exist in this study, and the meta-analysis without the distinction of surgical operation is an obvious one. In left-sided breast cancer patients with modified radical mastectomy (MRM), the target (i.e., chest wall) lies near the heart and LAD, as compared to those patients undergoing breast conservation surgery (BCS). Recently, a small sample study by Misra et al. showed that DIBH provided a similar percentage reduction in cardiopulmonary doses for both MRM and BCS. Significant reductions in mean heart dose were seen in both groups. Although lung and LAD doses were significantly reduced in MRM, lung dosimetric constraints were less frequently achieved in the subgroup with nodal radiation. Given that, we appeal to researchers to conduct more studies about the relationship between surgical methods and the benefits of DIBH technology, enabling more left-sided breast cancer patients to benefit from the development of precision medicine.

Apart from the distinction of surgical operation mode, other potential limitations are still prevalent in this study: (1) The data from the included studies were from the published articles instead of the original information of the individual patient; (2) all included articles are the retrospective studies, and the evidence level is lower than that of prospective randomized clinical trials; (3) the number of included studies is relatively small, especially for contralateral breast mean dose, which may cause bias results; (4) the heterogeneity of aggregated results were significant, and the random-effects model was applied to most indicators.

CONCLUSIONS

In summary, this study provides a large-scale and comprehensive meta-analysis between the dosimetric parameters of DIBH and FB for left-sided breast cancer. Although DIBH has no obvious advantage over FB in contralateral breast mean dose, it can significantly reduce the heart dose, LAD dose, ipsilateral lung dose, heart volume, and substantially increase the ipsilateral lung volume. This study suggests that DIBH may be more widely used in clinical practice soon because of its excellent dosimetric performance.

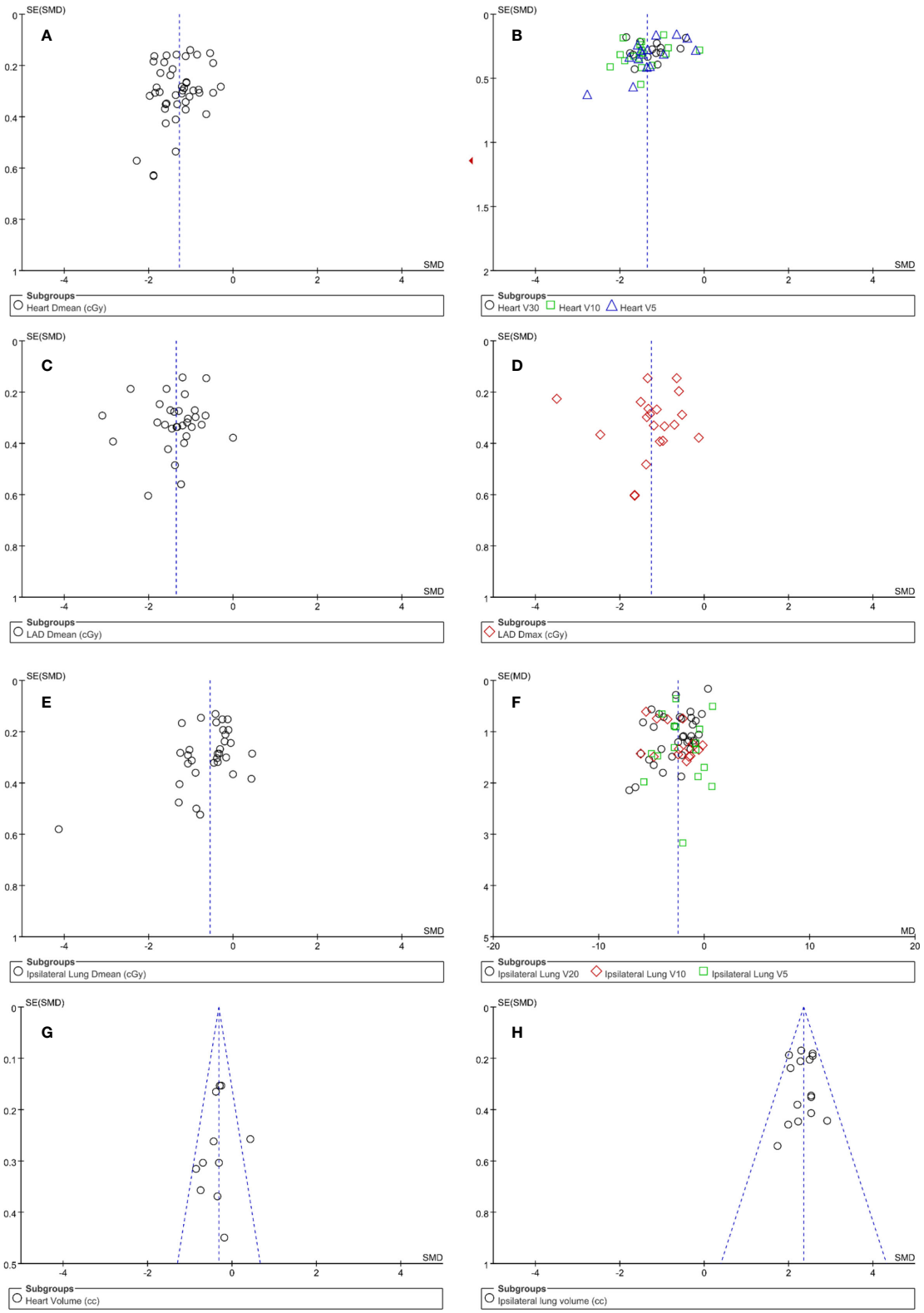

FIGURE 10 | Funnel plots for potential publication bias. Funnel plot analysis of heart dose **(A, B)**, LAD dose **(C, D)**, ipsilateral lung dose **(E, F)**, heart volume **(G)** and ipsilateral lung volume **(H)**.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

YKL, DY, and XZ: conceptualization. YL, WY, YZ, FT: data curation and original draft writing. YKL, YT, RH: statistical analysis. YL, JP, BH, RC: manuscript review and editing. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

This is a short text to acknowledge the contributions of specific colleagues, institutions, or agencies that aided the efforts of the authors.

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frontiers | Frontiers in Oncology

ORIGINAL RESEARCH
published: 28 June 2022
doi: 10.3389/fonc.2022.883679

Subclinical Left Ventricular Dysfunction Detected by Speckle-Tracking Echocardiography in Breast Cancer Patients Treated With Radiation Therapy: A Six-Month Follow-Up Analysis (MEDIRAD EARLY-HEART study)

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OPEN ACCESS

Edited by:
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GZA, Belgium

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Specialty section:
This article was submitted to Radiation Oncology, a section of the journal Frontiers in Oncology

Received: 25 February 2022
Accepted: 20 May 2022
Published: 28 June 2022

Citation:
Locquet M, Spoor D, Crijns A, van der Harst P, Eraso A, Guedea F, Fiuza M, Santos SCR, Combs S, Borm K, Mousseaux E, Gencer U, Frija G, Cardis E, Langendijk H and Jacob S (2022) Subclinical Left Ventricular Dysfunction Detected by Speckle-Tracking Echocardiography in Breast Cancer Patients Treated With Radiation Therapy: A Six-Month Follow-Up Analysis (MEDIRAD EARLY-HEART study). Front. Oncol. 12:883679. doi: 10.3389/fonc.2022.883679

Background: In the case of breast cancer (BC), radiotherapy (RT) helps reduce locoregional recurrence and BC-related deaths but can lead to cardiotoxicity, resulting in an increased risk of long-term major cardiovascular events. It is therefore of primary importance to early detect subclinical left ventricular (LV) dysfunction in BC patients after RT and to determine the dose–response relationships between cardiac doses and these events.

Methods: Within the frame of the MEDIRAD European project (2017–2022), the prospective multicenter EARLY-HEART study (ClinicalTrials.gov Identifier: NCT03297346) included chemotherapy naïve BC women aged 40–75 years and treated with lumpectomy and adjuvant RT. Myocardial strain analysis was provided using speckle-tracking echocardiography performed at baseline and 6 months following RT. A global longitudinal strain (GLS) reduction >15% between baseline and follow-up was defined as a GLS-based subclinical LV dysfunction. Individual patient dose distributions were obtained using multi-atlas-based auto-segmentation of the heart. Dose-volume parameters were studied for the whole heart (WH) and left ventricle (LV).

Results: The sample included 186 BC women (57.5 ± 7.9 years, 64% left-sided BC). GLS-based subclinical LV dysfunction was observed in 22 patients (14.4%). These patients had significantly higher cardiac exposure regarding WH and LV doses

compared to patients without LV dysfunction (for mean WH dose: 2.66 ± 1.75 Gy versus 1.64 ± 0.96 Gy, $p = 0.01$). A significantly increased risk of subclinical LV dysfunction was observed with the increase in the dose received to the WH [ORs from 1.13 (V_5) to 1.74 (D_{mean}); $p < 0.01$] and to the LV [ORs from 1.10 (V_5) to 1.46 (D_{mean}); $p < 0.01$]. Based on ROC analysis, the LV-V_5 parameter may be the best predictor of the short-term onset of subclinical LV dysfunction.

Conclusion: These results highlighted that all cardiac doses were strongly associated with the occurrence of subclinical LV dysfunction arising 6 months after BC RT. Whether measurements of GLS at baseline and 6 months after RT combined with cardiac doses can early predict efficiently subclinical events occurring 24 months after RT remains to be investigated.

Keywords: MEDIRAD, breast cancer, radiotherapy, cardiac dysfunction, dosimetry, strain imaging, EARLY-HEART cohort

INTRODUCTION

Breast cancer (BC) among women represents a public health challenge due to its rising incidence and its life-threatening consequences (1). Prescribed to reduce local recurrence and BC-related mortality, radiation therapy (RT) has widely demonstrated effectiveness in the treatment of BC (2). However, radiation-induced adverse effects in healthy tissues could occur. Cardiotoxicity resulting from incidental irradiation of the heart in BC patients is now better documented (3). Indeed, BC RT leads to an increased risk of long-term major adverse cardiovascular events (MACEs), mainly coronary heart diseases, as well as excess cardiovascular (CV) mortality rates (3, 4). Up to several decades, the relative risk of clinically significant cardiac events ranged from 1.2 to 3.6 after RT (5). Darby et al. (2013) showed an incidence of acute coronary events increased by 7.4% per Gray (Gy) of mean heart dose already within 5 years following RT, later confirmed by van den Bogaard et al. (2017) who found an incidence of 16.5% per Gy (6, 7) in the first 9 years. Other authors suggested a 0.04 (95% CI: 0.02–0.06) excess relative risk per Gy received at the whole heart (8). However, the asymptomatic phase between acute heart damage occurring early after RT and the longer-term onset of MACEs leads to an underrecognized CV risk during the clinical management of BC patients immediately following RT (9).

Therefore, early screening for subclinical CV changes following RT could prove beneficial for asymptomatic patients who could nevertheless have subclinical left ventricle (LV) dysfunction. According to the American Society of Echocardiography and the European Association of Cardiovascular Imaging, oncological cardiotoxicity is diagnosed when the left ventricular ejection fraction (LVEF) is reduced by ≥10% points to below 53% after RT (10). However, the myocardial deformation [i.e., global longitudinal strain (GLS), measured by two-dimensional (2D) speckle-tracking echocardiography] appeared to be an earlier marker of subclinical LV dysfunction. Specifically, strain imaging characterizes cardiac wall deformation considering speckles. Therefore, a reduction in LVEF reflects late and advanced myocardial injury in relation to substantial cardiac damage (11). Therefore, the measurement of GLS appears to be more sensitive and relevant for detecting early LV dysfunction before the onset of LVEF deterioration, and in identifying a population at greater risk of longer-term CV morbidity and mortality (12, 13).

The ability of GLS to detect cardiotoxicity early has been little investigated among BC patients treated with RT. Some studies have shown that a statistically significant reduction of the GLS can be detected in BC women from a few weeks to 12 months following RT (14–17). However, it remains to be determined whether the observed reduction can be considered clinically relevant. Negishi et al. suggested that a reduction of GLS >15% compared with baseline appears to be clinically meaningful to highlight post-RT cardiotoxicity, but this GLS cutoff limit was scarcely applied in onco-cardiology research (18). Moreover, few studies investigated the dose-dependent relationship between RT and changes in GLS. In 2019, Walker et al. investigated the clinical relevance of the reduction of GLS in 79 BC patients included in the BACCARAT study (14) by defining a subclinical LV dysfunction as a relative reduction of GLS >10%. A dose–response relationship was observed, and the risk of subclinical LV dysfunction was increased by 37% per 1 Gy of mean heart dose. Nevertheless, the association was no longer statistically significant after adjustment for age, body mass index (BMI), hypertension, hypercholesterolemia, and endocrine therapy, and the study suffered from its small size and statistical power (19).

Therefore, within the frame of the European MEDIRAD project, the multi-center EARLY-HEART cohort study was designed to investigate early cardiac changes arising after BC RT in the largest population ever studied, using three approaches based on echocardiography, cardiac MRI and heart CT, and computed tomography coronary angiography (20). The present manuscript originally focuses on the specific purpose of evaluating the impact of RT (using individual patient dosimetry) on subclinical LV function changes (using speckle-tracking echocardiography) occurring in the first 6 months after BC RT. This study will open many research possibilities to find

markers of early subclinical LV dysfunction potentially predicting long-term MACEs.

MATERIALS AND METHODS

Reporting

The guidelines proposed by the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement were applied to the manuscript (21).

The EARLY-HEART Study Design

As part of the MEDIRAD project (http://www.medirad-project.eu/), the multi-center EARLY-HEART study was launched in 2017. This observational study consisted of the prospective follow-up of a cohort of BC patients treated with RT over two time points post-RT (i.e., 6 and 24 months). The detailed protocol has already been described elsewhere and registered at ClinicalTrials.gov (identifier NCT03297346) (20).

The main goal of the EARLY-HEART study was to explore the relevance of several cardiac biomarkers to early identify radiation-induced subclinical dysfunction in women with unilateral left- or right-sided BC. For this purpose, both imaging biomarkers (i.e., echocardiography, computed tomography coronary angiography, and magnetic resonance) and blood-circulating biomarkers were assessed at baseline and at 6 months following RT. The current article focuses on the assessment of subclinical dysfunction post-RT using 2D speckle-tracking echocardiography at the 6-month follow-up.

Patients were included from 5 European investigation centers: the Clinique Pasteur (Toulouse, France) for the Institut de Radioprotection et de Sûreté Nucléaire (IRSN; Fontenay-aux-Roses, France), the Universitair Medisch Centrum Groningen UMCG; Groningen, Netherlands), the Klinikum Rechts der Isar der Technischen Universität München (TUM-MED; Munich, Germany), the Institut Català d'Oncologia (ICO; Girona, Spain), and the Centro Cardiovascular da Universidade de Lisboa (CCUL; Lisbon, Portugal).

Breast Cancer Women Population

All women aged 40–75 years with histologically diagnosed unilateral left- or right-sided stage I–III invasive adenocarcinoma of the breast or ductal carcinoma *in situ* (DCIS) and treated with adjuvant RT after breast-conserving surgery in one of the 5 investigating centers could be included. In addition, women had to be chemotherapy naïve. Non-inclusion criteria were previous thoracic or mediastinal radiation, previous CV diseases, and current pregnancy and/or lactation. Abnormal cardiac imaging exams after inclusion were considered as dismissal criteria. Specifically for echocardiography, an LVEF <50%, suggesting an alteration of the cardiac function before RT, was set as a dismissal criterion.

Radiation Therapy Treatment

All patients underwent adjuvant radiotherapy following the lumpectomy. According to the center, three-dimensional conformal radiotherapy (3D-CRT), volumetric modulated arc therapy (VMAT), and/or fixed-field intensity-modulated radiotherapy (IMRT) was performed.

Different fractionation schedules were used according to patient and center specificities: mainly 25 fractions/50 Gy following a standard protocol or 15 fractions/40.5 Gy following a hypofractionated protocol. A boost dose was delivered to the tumor site in some patients (with a maximum of 14.49 Gy administered). Deep inspiring breath-holding was recommended in some patients with a heart close to the anterior chest wall and in all left-sided patients followed at the UMCG center. The patient treatment was normalized and optimized according to the statement of the International Commission on Radiation Units and Measurements (ICRU) and in compliance with QUANTEC dose constraints (5).

Individual Patient Dosimetry

Cardiac structure delineation was performed centrally by the UMCG using multi-atlas based automatic segmentation of the heart and its substructures previously published by Spoor et al. (22). This technique reduces inter-observer variability during the delineation of cardiac volumes. Two contoured cardiac structures were considered in our analysis: the whole heart (WH) and the left ventricle (LV), their relevance being highlighted in previous research. The exact planned radiation dose was reconstructed from the delineated volumes and three-dimensional dose-volume parameters were obtained for each patient. In the current analysis, mean dose (D_{mean}, in Gy), minimum dose (D_{min}, in Gy), and maximum dose (D_{max}, in Gy) were studied as well as relative volumes of the WH and LV receiving at least 5 Gy (V_5, in %) and 20 Gy (V_{20}, in %), both suggested as good prognostic parameters of cardiac complications (14, 23).

Cardiac Examinations

Two-dimensional speckle-tracking trans-thoracic echocardiography, a recent semi-automated imaging technique, was performed before RT and at the 6-month follow-up. The level of deformation between systole and diastole is expressed in percentage and will be negative in the presence of shortening (24). Subsequently, longitudinal shortening will engender negative values. A weakened myocardium is described by a reduced systolic function followed by a smaller decline between systole and diastole. The strain value is then reduced and closer to zero (24). Left lateral decubitus position was required for the exam performed by a trained and qualified cardiologist or technician. Different measurement techniques were used between the different institutes (Siemens, Philips, or General Electric). Different software was used to calculate strain values. LVEF was determined using Simpson's biplane method during three sets of measurements (mean was reported) (25). Other conventional measurements have been collected: left ventricular end-diastolic volume, left ventricular end-systolic volume, E/A wave ratio, tricuspid annular plane systolic excursion, tricuspid annular S wave, left ventricular outflow tract diameter, left ventricular outflow tract velocity time integral, heart rate, and cardiac output. By tracking movements of myocardial speckles

occurring during 3 cardiac cycles including an apical 4-, 3-, and 2-chamber view, the 2D speckle-tracking echocardiography also provided systolic strain values (26). GLS (%) and GLS rate (s^{-1}) have been recorded. A >15% relative percentage reduction from the initial GLS value was considered a clinically relevant marker of subclinical LV dysfunction as suggested in 2016 by the European Society of Cardiology (10). Based on LVEF, subclinical LV dysfunction was defined according to Cancer Therapy-Related Cardiac Dysfunction (CTRCD) definition for patients with a reduction in LVEF ≥10% from baseline to a final value less than 53% after RT (27). Images with poor echogenicity were excluded as well as patients without echocardiography imaging available at the two time points. All ultrasound data were collected at each center by operators blinded to all other clinical data, including radiotherapy treatment modalities.

Non-Radiation CV Risk Factors

In addition to BC treatment characteristics, information on clinical patients' characteristics were collected at baseline, particularly the CV risk factors such as age, BMI, smoking status, hypertension, diabetes, cholesterol, menopausal status, and statin consumption.

Statistical Analysis

All quantitative variables were expressed as mean (μ) ± standard deviation (SD). Group comparisons were carried out using a *t*-test in case of normal distribution (checked using the Shapiro–Wilk test) or a nonparametric Wilcoxon–Mann–Whitney test in case of skewed distribution. Qualitative variables were reported in absolute (*n*) and relative (%) frequencies and were compared using χ^2 or Fisher's exact tests. Paired Wilcoxon signed-rank tests were applied to assess changes in echocardiography parameters before RT and 6 months post-RT. The impact of baseline characteristics (i.e., age, smoking status, hypertension, obesity, diabetes, total cholesterol level, and hormonotherapy) on the risk of subclinical LV dysfunction was explored using a binary logistic regression yielding odds ratio (OR) and the 95% confidence interval (95% CI). The relationship between dose-volume parameters and subclinical LV dysfunction was also investigated using univariate (crude model) and multivariate (adjusted model) binary logistic regressions. An adjustment was made for age, smoking status, hypertension, total cholesterol level, and hormonal therapy. Obesity and diabetes were not included in the model to avoid the strong correlation with total cholesterol levels. To determine which dose-volume parameter best discriminates between BC patients at risk of subclinical LV dysfunction and those not at risk, areas under the curve were obtained using receiver operating characteristic analysis (AUROC). An AUROC between 0.5 (no discriminative power) and 1 (perfect discriminative power) is essential for clinical testing (28). The AUROC values of the different dose-volume parameters were statistically compared using the method of Delong et al. (29). Optimal cutoffs were calculated according to Youden's index. The 5% critical threshold was set to consider statistically significant results. In regression models, because of multiple testing, the significance level was further corrected in 0.05/k (Bonferroni correction). All analyses were performed using R version 4.0.3 software.

RESULTS

Description of the Studied Population

The 5 European centers included a total of 258 BC patients. For the present study based on echocardiography parameters, 186 BC patients were analyzed, 72 being excluded due to the absence of paired echocardiography data available (i.e., before RT and 6 months post-RT). A detailed flowchart is available in **Figure 1**.

The 186 BC women had a mean age of 57.5 ± 7.9 years. Baseline characteristics of the 186 BC patients are described in **Table 1**. Left-sided BC represented 64% of the sample, 14.5% were obese, a few BC women were affected by diabetes mellitus (4.3%), and more than half were non-smokers (52.7%). A large sample suffered from an invasive (78.0%) grade 2 (51.9%) carcinoma. Patients were mainly treated by 3D-CRT (60.2%), with a 15 fraction/40.05 Gy (33.9%) protocol; 35.5% received a boost, and 65% received hormonal therapy.

Cardiac Radiation Dosimetry

The cardiac dose-volume parameters are reported in **Table 2**. D_{mean} to WH and D_{mean} to LV dose were 1.76 ± 1.16 Gy and 2.09 ± 1.91 Gy, respectively, with higher dose-volume parameters for left-sided BC than for right-sided BC (p-value < 0.001).

Echocardiography Parameters

Conventional echocardiography parameters at baseline and 6 months post-RT are shown in **Table 3**. No significant changes were shown between those parameters before and after RT (all p-values >0.05). LVEF-based LV dysfunction defined by a ≥10% reduction in LVEF from baseline to <53% after RT was found in 6 patients (3.2% of the sample).

Regarding the strain imaging, by considering GLS and GLS rate as continuous variables, no significant changes were observed between baseline and 6 months post-RT (all p-values >0.05) (**Table 4**). Subclinical LV dysfunction, defined as a relative reduction of GLS >15%, was observed in 11.8% of the total sample (i.e., 22 patients). Among the 22 women with subclinical LV dysfunction, 4 had a right-sided BC (18.2%) and 18 had a left-sided BC (81.8%) (p-value = 0.21). Among the 6 patients with LVEF-based LV dysfunction, 5 patients (83.3%) had a reduction of GLS >15%.

Relationships Between a Reduction of GLS >15% and Clinical or Radiation Parameters

The impact of baseline characteristics on the risk of a reduction of GLS >15% at the 6-month follow-up was analyzed (**Table 5**). Higher total cholesterol levels increased the risk of subclinical LV dysfunction (OR = 1.02 [1.01–1.03]). However, no other usual CV risk factors were associated (all p-values >0.05) with GLS reduction. Parameters of the RT protocol also affected the onset of a subclinical LV dysfunction: the RT protocol (i.e., fraction ×

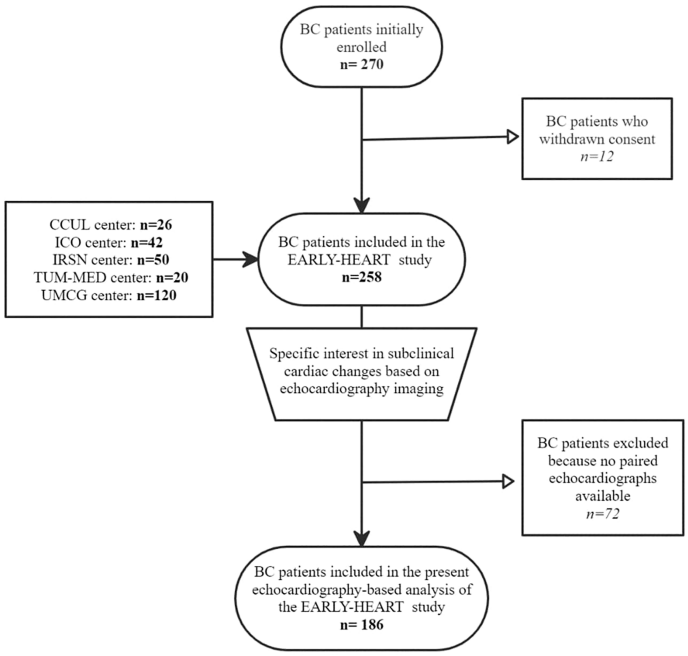

FIGURE 1 | Flowchart of BC patient inclusion and exclusion in the EARLY-HEART study based on echocardiography.

total dose) increased the risk by 4.32-fold (95% CI of the OR: 1.33–16.8), irradiation of lymph nodes by 5.55-fold (95% CI of the OR: 1.27–23.0), and a boost by 2.83-fold (95% CI of the OR: 1.09–8.32) (all *p*-values <0.05).

Comparisons between dose-volume parameters obtained for the WH and the LV were performed between patients with or without relative reduction of GLS >15%. A significantly higher mean dose was observed in patients with a relative reduction of GLS >15% (**Figure 2**).

The same observation was made regarding V_5 (%) and V_{20} (%) (**Figure 2**). Further analysis was undertaken to determine the magnitude of the association between dose-volume parameters and the reduction of GLS >15% (**Table 6**).

All dose-volume parameters to WH and LV (D_{mean}, V_5, and V_{20}) were significantly associated with a reduction of GLS >15% (all *p*-values <0.001), except for D_{min} and D_{max} (after adjustment). For both cardiac structures, the associations remained significant after adjustment for covariates and multiple statistical testing (all *p*-values <0.05). In the adjusted model, each increase of 1 Gy of D_{mean} to WH increased the risk of a reduction of GLS >15% by 74% and each increase of 1 Gy of D_{mean} to LV increased the risk by 46%. Moreover, each additional percent of V_5 and V_{20} increased the risk of subclinical LV dysfunction by 13% and 39% (WH) and by 10% and 20% (LV), respectively. Sensitivity analysis had been undertaken using a reduction of GLS >10% to define a subclinical LV dysfunction (**Supplementary Table 1**). A reduction of GLS >10% was highlighted in 36 patients (i.e., 23.5% of the total sample). Similar conclusions were drawn using this cutoff. **Supplementary Table 2** also highlights the consistency of our results when applying a one-way sensitivity analysis omitting one center at a time.

Table 6 provides information about the ability of dose-volume parameters to early identify BC patients at risk of subclinical LV dysfunction occurring 6 months following RT. All parameters showed an AUROC value higher than the point with no discriminant power (i.e., 0.500) except for D_{min}. The highest AUROC value was observed for V_5 regardless of the cardiac structure (i.e., 0.813 for WH and 0.815 for LV). Also, AUROC values of V_{20} (i.e., 0.804 for WH and 0.808 for LV) showed a very satisfactory discriminative power. However, the AUROC values of all the dose-volume parameters did not differ between them (all *p*-values >0.05). Therefore, no dose-volume parameter statistically performed better than another. In addition to the dose–response relationship showing the relevance of heart dose for GLS reduction with a risk gradually increasing with higher doses, we evaluated the optimal cutoff of dose parameters to predict the risk of subclinical LV dysfunction-based ROC analysis. D_{mean} >2.74 Gy to the WH was the mean dose from which the classification of our patients between the two groups (i.e., with or without subclinical LV dysfunction) was the most accurate. Regarding LV, a D_{mean} >3.1 Gy was established. For V_5, the threshold was set at >5.2% (WH) and >8.4% (LV).

DISCUSSION

Designed to early identify cardiotoxicity in BC women treated with RT, the EARLY-HEART study suggested a strong relationship between cardiac absorbed dose and the occurrence of subclinical

TABLE 1 | Baseline characteristics of the 186 BC women from the EARLY-HEART cohort included in the echocardiography-based analysis.

Characteristics	μ ± SD or *n* (%)
Clinical and cardiovascular data	
Age, years	57.5 ± 7.9
Body mass index, kg/m²	25.5 ± 4.1
Menopausal status, yes	137 (74.9)
Onset of menopause, years	11.4 ± 7.4
Cardiovascular treatment, yes	23 (12.4)
Statins prescription, yes	20 (10.7)
Obesity, yes	27 (14.5)
Total cholesterol, mg/dl	209.7 ± 47.2
Triglycerides, mg/dl	106 ± 56.1
Diabetes mellitus, yes	8 (4.3)
Hypertension, yes	41 (22.0)
Smoking status	
No	98 (52.7)
Former	58 (31.2)
Current	30 (16.1)
Former or current smoker, pack-year	14.3 ± 12.3
Breast cancer information	
Laterality, left	119 (64.0)
Invasive breast carcinoma, yes	145 (78.0)
Breast carcinoma *in situ*, yes	101 (54.3)
Grade of breast cancer	
Grade 1	66 (36.1)
Grade 2	95 (51.9)
Grade 3	22 (12.0)
Radiotherapy protocol	
Type of radiotherapy	
3D-CRT	112 (60.2)
IMRT	2 (1.1)
VMAT	72 (38.7)
Fraction/total RT dose	
15/40.05 Gy	63 (33.9)
16/42.56 Gy	36 (19.3)
20/47 Gy	25 (13.4)
2 5/50 Gy	39 (21.0)
Lymph node radiation, yes	11 (5.9)
Breath-hold RT, yes	66 (35.5)
Boost, yes	95 (51.1)
Total boost dose, Gy	11.9 ± 1.9
Other breast cancer treatment	
Hormonotherapy	
No	65 (35.1)
Tamoxifen	72 (38.9)
Aromatase inhibitors	48 (25.9)

BC, breast cancer; SD, standard deviation.

TABLE 2 | Dose-volume parameters for the whole heart and left ventricle.

	Whole BC patients (*n* = 186)		Left-sided BC patients (*n* = 119)		Right-sided BC patients (*n* = 67)		
Dosimetry	**μ ± SD**	**Range**	**μ ± SD**	**Range**	**μ ± SD**	**Range**	***p*-value**
Whole heart							
D_{mean} (Gy)	1.76 ± 1.12	0.14–6.76	2.21 ± 1.17	0.14–6.76	0.97 ± 0.34	0.28–2.02	<0.0001
D_{min} (Gy)	0.33 ± 0.25	0.00–1.20	0.38 ± 0.28	0.00–1.20	0.25 ± 0.16	0.00–0.80	<0.0001
D_{max} (Gy)	23.6 ± 18.5	0.88–55.4	33.9 ± 15.0	0.88–55.4	5.41 ± 5.07	2.16–29.9	<0.0001
V_5 (%)	3.80 ± 5.66	0.00–31.3	5.84 ± 6.21	0.00–31.3	0.21 ± 0.72	0.00–5.20	<0.0001
V_{20} (%)	1.03 ± 1.95	0.00–12.2	1.63 ± 2.25	0.00–12.2	0.01 ± 0.06	0.00–0.50	<0.0001
Left ventricle							
D_{mean} (Gy)	2.09 ± 1.91	0.04–8.18	2.97 ± 1.87	0.07–8.18	0.53 ± 0.30	0.04–1.60	<0.0001
D_{min} (Gy)	0.50 ± 0.31	0.00–1.61	0.64 ± 0.28	0.00–1.61	0.26 ± 0.17	0.00–0.83	0.005
D_{max} (Gy)	18.2 ± 18.4	0.23–55.2	27.9 ± 16.5	0.25–55.2	1.13 ± 0.70	0.23–5.35	<0.0001
V_5 (%)	5.34 ± 8.12	0.00–36.8	8.38 ± 8.84	0.00–36.8	0.00 ± 0.00	0.00–0.00	<0.0001
V_{20} (%)	1.49 ± 3.16	0.00–14.2	2.35 ± 3.71	0.00–14.2	0.00 ± 0.00	0.00–0.00	<0.0001

BC, breast cancer; SD, standard deviation; Gy: Gray.

TABLE 3 | Description of conventional echocardiography parameters before RT and RT+6 months.

Echocardiography parameters	Before RT	RT+6 months	*p*-value
Left ventricular ejection fraction, %	62.3 ± 6.1	61.5 ± 6.6	0.08
Left ventricular end-diastolic volume, ml	77.4 ± 18.8	76.9 ± 19.2	0.90
Left ventricular end-systolic volume, ml	30.1 ± 10.2	30.1 ± 9.6	0.67
E/A wave ratio	1.05 ± 0.52	1.03 ± 0.31	0.97
Tricuspid annular plane systolic excursion, cm	3.21 ± 4.10	2.39 ± 0.33	0.15
Tricuspid annular S wave, cm/s	13.29 ± 2.49	13.47 ± 2.52	0.32
Left ventricular outflow tract diameter, mm	20.13 ± 3.87	19.93 ± 2.26	0.36
Left ventricular outflow tract velocity time integral, cm	22.5 ± 4.79	22.55 ± 4.10	0.56
Heart rate, beats per minute	68.1 ± 9.04	68.6 ± 11.4	0.82
Cardiac output, L/min	4.79 ± 2.50	4.29 ± 1.62	0.17

RT, radiation therapy.

TABLE 4 | Global longitudinal strain and strain rate parameters before RT and at RT+6 months.

	GLS (%)	GLS rate (s^{-1})
Before RT	−19.4 ± 3.2	−1.08 ± 0.20
RT+6 months	−19.2 ± 3.6	−1.09 ± 0.34
p-value	0.82	0.13

RT, radiation therapy.

LV dysfunction at 6 months following RT based on >15% reduction in GLS estimated by echocardiography.

The mean value of GLS in the whole population did not significantly decrease from baseline to 6 months post-RT. Other authors previously showed significant GLS changes after BC RT, with a mean reduction of the GLS following RT of 5% in Erven et al., 6% in Walker et al., and 7.9% in Trivedi et al. (follow-up from 3 to 12 months) (14, 16, 17). In these studies, significant changes were highlighted in left-sided BC only.

GLS damage was further studied as a relative change in each individual and from a clinical perspective (10). A binary clinical endpoint of subclinical LV dysfunction was set by categorizing BC patients with or without reduction of GLS >15% as previously suggested in order to be largely beyond the possible errors related to the accuracy and reproducibility of measurements (18). Among the 186 women, 22 presented a subclinical LV dysfunction (11.8%). The prevalence of subclinical LV

TABLE 5 | Univariate logistic regressions exploring the relationship between baseline characteristics and a relative reduction of GLS >15% occurring 6 months after BC RT.

Characteristics	OR (95% CI)	*p*-value
Clinical and cardiovascular data		
Age, years	1.00 (0.94–1.06)	0.99
Menopause, yes	0.84 (0.32–2.57)	0.77
Cardiovascular treatment, yes	0.29 (0.02–1.56)	0.99
Obesity, yes	1.05 (0.23–3.51)	0.94
Total cholesterol, mg/dl	1.02 (1.01–1.03)	0.02
Triglycerides, mg/dl	1.00 (0.99–1.01)	0.66
Diabetes mellitus, yes	4.23 (0.53–27.1)	0.12
Hypertension, yes	0.89 (0.24–2.63)	0.84
Smoking status, yes	1.01 (0.90–1.08)	0.97
Breast cancer information		
Laterality, left	2.30 (0.80–8.34)	0.31
Hormonotherapy	1.23 (0.47–3.63)	0.69
No (reference)	1	0.49
Tamoxifen	0.64 (0.17–2.28)	0.17
Aromatase inhibitors	2.15 (0.73–6.79)	
Radiotherapy protocol		
3D-CRT, yes	2.15 (0.74–7.78)	0.19
VMAT, yes	0.47 (0.13–1.37)	0.20
Fraction/total RT dose	1	0.90
15/40.05 Gy (reference)	1.10 (0.20–5.33)	0.06
16/42.56 Gy	4.40 (0.90–21.7)	0.02
20/47 Gy	4.32 (1.33–16.8)	
25/50 Gy		
Lymph node radiation, yes	5.55 (1.27–23.0)	0.02
Breath-hold RT, yes	0.33 (0.09–0.95)	0.04
Boost, yes	2.83 (1.09–8.32)	0.04

RT, radiation therapy; GLS, global longitudinal strain; BC, breast cancer; Gy, Gray; 3D-CRT, three-dimensional conformal radiotherapy; VMAT, volumetric modulated arc therapy.

dysfunction was slightly higher when applying the cutoff of 10% (19.3%). Although not negligible, these two rates were lower than those obtained in other studies (applying the 10% cutoff) where they ranged from 27.5% to 46.8% (14, 16, 17). The high proportion of right-sided BC, cardiac dose differences, and the chemotherapy-naïve status of BC women in our study may explain this lower rate. Fourati et al., using similar study criteria, also obtained a lower prevalence rate of cardiac dysfunction (6.8%) (30) (i.e., 1.76 ± 1.12 Gy versus in our study versus 2.8 Gy of mean dose in the study of Fourati et al.; 22% of left-sided BC versus 42% of right-sided BC).

The EARLY-HEART study robustly showed a relationship between dose-volume parameters and an increased risk of subclinical LV dysfunction (adjusted ORs ranging from 1.13 [1.05–1.23] (V_5) to 1.74 [1.20–2.61] (D_{mean}) for the WH structure and from 1.10 [1.05–1.17] (V_5) to 1.46 [1.17–1.87] (D_{mean}) for the LV structure). The magnitude of the association was consistent with previous studies (or even stronger): OR = 1.37 [1.01–1.86] in Walker et al. and OR = 1.04 [1.01–1.06] in Fourati et al. (both analyzing relationship between D_{mean} and a reduction in GLS >10%) (30, 31). Furthermore, three parameters were able to properly distinguish BC women at risk or not of a reduction of GLS>15%, 6 months after RT: D_{mean}, D_{max}, and $V_{5.}$ The lowest AUROC was 0.765 for D_{max} (WH) and the highest AUROC was 0.815 for V_5 (LV). Then, V_5, a dose-volume parameter, seemed highly relevant, as previously shown by other studies (7, 14). Indeed, van den Bogaard et al. showed that V_5 (LV) was the best predictor of acute coronary events (HR = 1.016 [1.002–1.030], *p*-value = 0.016). However, the mean heart dose remains currently the most widely used predictor of cardiotoxicity (31). Furthermore, intrinsic to our sample, the threshold of 3 Gy for D_{mean}, previously identified by Erven et al. (17), was also highlighted. Indeed, a D_{mean} of 2.74 Gy (WH) or a V_5 >5.2% (WH) should not be exceeded to prevent the CV risk. The threshold of 3 Gy for D_{mean}, previously identified by Erven et al. (17), was also highlighted. Some impactful studies (e.g., Darby et al., showing a dose–response relationship between acute coronary events and mean heart dose) incited RT protocols to evolve to limit the risk of MACE and cardiac doses (e.g., breath-hold, VMAT, and hypofractionation can reduce cardiotoxicity) (6). Proton therapy may also be applied for patients still at increased risk. However, our study combining different techniques of RT showed that some patients remained in dose ranges that should be considered with caution (e.g., D_{mean} of 3 Gy to LV). Vigilance must be brought to this specific point, especially in randomized controlled trials where a systemic assessment of radiation-induced cardiotoxicity should be investigated as a clinical endpoint.

Our study was the first to demonstrate, with sufficient statistical power, a dose-dependent relationship between early cardiotoxicity defined using the stringent and recommended criterion of a reduction of GLS >15% and a wide range of doses absorbed (inclusion of both right- and left-sided BC

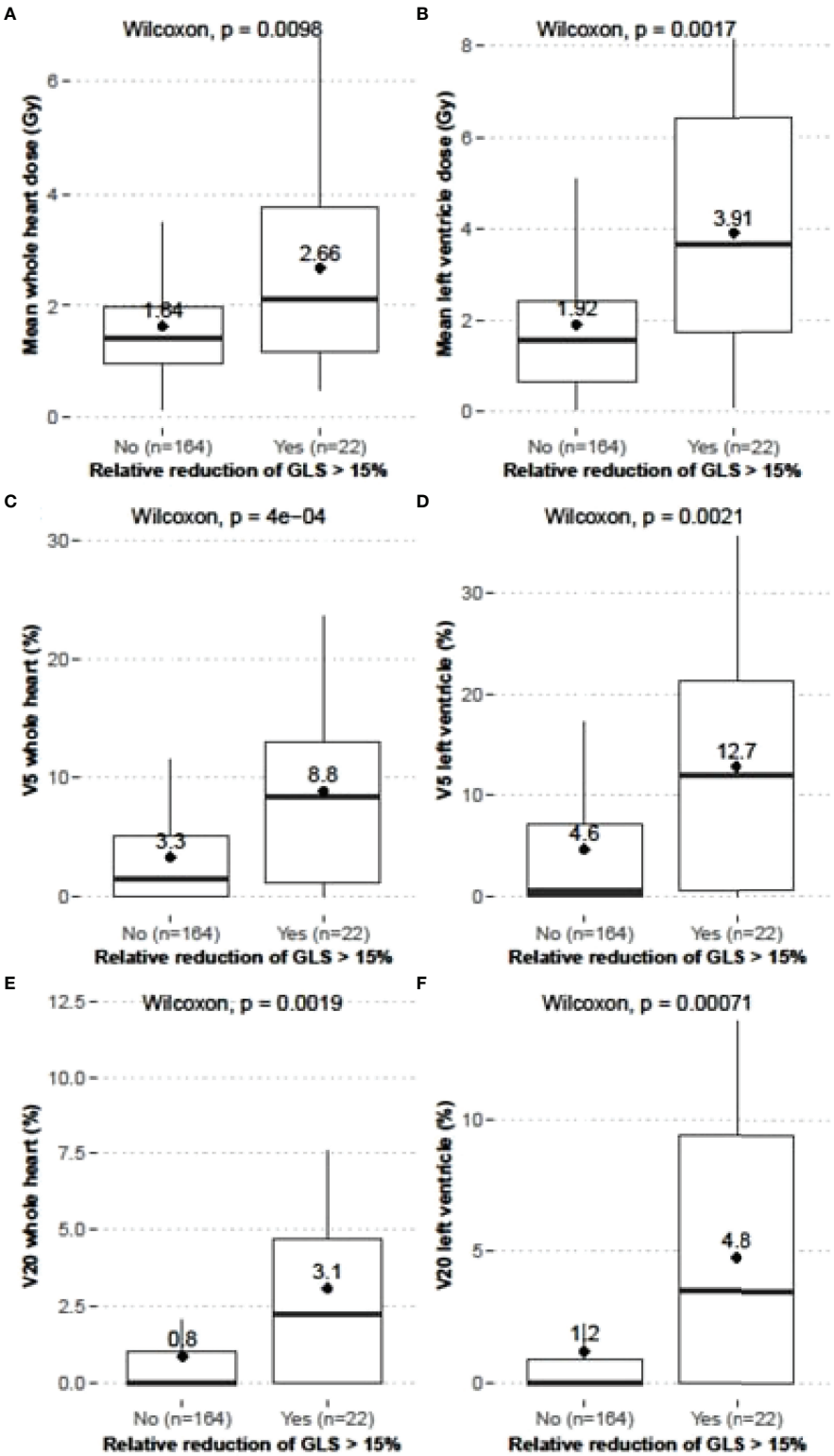

FIGURE 2 | Comparisons of dose-volume parameters between BC patients with or without a reduction of GLS >15%; D_{mean} to WH **(A)** and LV **(B)** (Gy), V_5 of WH **(C)** and LV **(D)**, and V_{20} of WH **(E)** and LV **(F)**; mean values: numerical and ◆.

TABLE 6 | Relationship between dose-volume parameters and a relative reduction of GLS >15% occurring 6 months after BC RT highlighted by binary logistic regressions and ROC analyses (n = 186 patients from the EARLY-HEART cohort).

Dosimetry	Crude OR (95% CI)	*p*-value	Adjusted[a] OR (95% CI)	*p*-value	Adjusted[a] AUROC (95% CI)	*p*-value
Whole heart						
D_{mean} (Gy)	1.85 (1.31–2.68)	0.0005	1.74 (1.20–2.61)	0.005	0.794 (0.688–0.902)	0.02
D_{min} (Gy)	0.24 (0.02–1.86)	0.20	0.36 (0.02–3.49)	0.40	0.575 (0.492–0.655)	0.28
D_{max} (Gy)	1.04 (1.01–1.07)	0.0009	1.03 (0.99–1.06)	0.02[b]	0.765 (0.651–0.878)	0.004
V_5 (%)	1.14 (1.06–1.23)	0.0004	1.13 (1.05–1.23)	0.002	0.813 (0.712–0.914)	0.001
V_{20} (%)	1.47 (1.21–1.81)	0.0001	1.39 (1.13–1.75)	0.003	0.804 (0.703–0.906)	0.001
Left ventricle						
D_{mean} (Gy)	1.51 (1.23–1.88)	0.00008	1.46 (1.17–1.87)	0.003	0.807 (0.704–0.909)	0.004
D_{min} (Gy)	3.59 (0.80–16.33)	0.09	5.11 (0.91–3.30)	0.07	0.629 (0.457–0.706)	0.06
D_{max} (Gy)	1.04 (1.01–1.06)	0.005	1.03 (1.01–1.06)	0.02[b]	0.770 (0.660–0.878)	0.003
V_5 (%)	1.10 (1.05–1.16)	0.0001	1.10 (1.05–1.17)	0.001	0.815 (0.717–0.914)	0.005
V_{20} (%)	1.26 (1.12–1.42)	0.00009	1.20 (1.07–1.37)	0.003	0.808 (0.701–0.909)	0.004

[a]*Model adjusted for age, smoking status, hypertension, total cholesterol level, and hormonotherapy.*
[b]*No longer after Bonferroni correction for multiple tests (significant threshold: α/k).*

patients). Its prospective design allowed us to include only BC women without baseline overt CV diseases and chemotherapy and to control CV risk factors, making the results on the observation of an early subclinical LV dysfunction induced by RT more robust.

However, our study had some limitations. The interpretation of the present results must be made with knowledge of these. First, our sample of BC women was limited by strict inclusion criteria. Further studies should include a larger representation of BC patients treated with RT only (e.g., risk in younger and older BC patients, risk in patients with or without previous CV diseases, and risk according to regional specificities). Likewise, the lower proportion of left-sided BC patients in our EARLY-HEART population compared to other studies could impact the observed change in mean GLS, which was not significant. Moreover, inter-observer (i.e., different cardiologists) and inter-operator (i.e., different vendors) imprecisions cannot be excluded to explain the absence of a statistically significant decrease in mean GLS in our study even if the good reproducibility of the strain measure using echocardiography was established (32). Indeed, inter-operator relative mean errors ranged from 5.4% to 11.0% when inter-observer relative mean errors varied from 1.9% to 11.3%. These values of errors remained lower than that observed for LVEF and other conventional echocardiography parameters (32). Our one-way sensitivity analysis omitting one center at a time reduced this potential bias and showed the robustness of our findings. Finally, although the current results were adjusted for baseline CV risk factors, it cannot be ruled out that other confounding factors could impact the observed association (e.g., parental history of CV diseases, sedentary habits, and nutritional habits).

In the future, it remains to be investigated whether the occurrence of subclinical LV dysfunction observed at RT+6 months is declining, maintaining, or improving in the longer term. The specific interest in echocardiography data imaging from the EARLY-HEART study will be further studied to determine which specific segments of the longitudinal strain (i.e., basal, mid, or apical) could be the most affected by dose-volume parameters. Indeed, Tuohinen et al. recently showed that the dose absorbed at the level of the apical region of the anterior wall of the LV was linked to a significantly higher deterioration of the GLS than in other locations (15). Furthermore, the same research team recently showed that diastolic strain rate was an earlier predictor of dysfunction than systolic LV strain rate (23), which could be of interest knowing that diastolic function is involved in diffuse fibrosis following RT.

CONCLUSION

The present analysis of BC women from the EARLY-HEART study showed that the cardiac doses absorbed during RT were strongly associated with the occurrence of a subclinical LV dysfunction at 6 months after RT. Therefore, primary and secondary CV health prevention could be beneficial at this early asymptomatic phase to reduce long-term CV complications. These findings already suggest the potential relevance of an early screening of BC patients treated with RT to eventually early implement cardioprotective actions during RT by limiting the dose absorbed by the heart as much as possible.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The study protocol and related amendments received approval from the competent ethics authority of each center involved (France: Comité de Protection des Personnes Sud-Ouest IV, ID: CPP2015/66/2015-A00990-69–R1, and Agence Nationale de Sécurité des Médicaments, ID: 150873B-12; the Netherlands: Medisch Ethische Toetsingscommissie van het Universitair

Medisch Centrum Groningen [METc UMCG], ID: METc 2017/379, NL62360.042.17; Germany: Ethikkomission der Technischen Universität München, ID: 235/17 S; Spain: Comitè d'Etica d'Investigatio CEAi GIRONA, ID: EARLY HEART v1.1 05/07/2017 i FIP v1.3; Portugal: Comissao de Ética do Centro Hospitalar Lisboa Norte e do Centro Académico de Medicina de Lisboa [CHLN e CAML], ID: 257/2017). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conceptualization: SJ, AC, HL, AE, SS, KB, GF, and EC. Methodology: SJ, HL, GF, and EC. Software: ML. Validation: ML and SJ. Formal analysis, ML and SJ. Investigation: SJ, AC, ML, DS, FG, MF, SS, SC, KB, EM, and UG. Resources: GF and EC. Data curation: ML, EM, UG, SJ, and DS. Writing—original draft preparation: ML and SJ. Editing: all authors. Supervision: EC, GF, and HL. All authors contributed to the article and approved the submitted version.

FUNDING

The European Community's Horizon 2020 Programme supported the EARLY-HEART study conducted in the frame of the MEDIRAD - Implications of Medical Low Dose Radiation Exposure - project spanning from 2017 to 2021 granted by the Euratom Research and Training Programme 2014-2014 under agreement No. 755523.

ACKNOWLEDGMENTS

The authors thank all the MEDIRAD-EARLY-HEART team and all the patients for their precious contribution.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.883679/full#supplementary-material

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frontiers | Frontiers in Oncology

ORIGINAL RESEARCH
published: 04 July 2022
doi: 10.3389/fonc.2022.892882

Association Between Cardiac Radiation Exposure and the Risk of Arrhythmia in Breast Cancer Patients Treated With Radiotherapy: A Case–Control Study

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OPEN ACCESS

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Specialty section:
This article was submitted to Radiation Oncology, a section of the journal Frontiers in Oncology

Received: *09 March 2022*
Accepted: *02 June 2022*
Published: *04 July 2022*

Citation:
Errahmani MY, Locquet M, Spoor D, Jimenez G, Camilleri J, Bernier M-O, Broggio D, Monceau V, Ferrières J, Thariat J, Boveda S, Kirova Y, Loap P, Langendijk JA, Crijns A and Jacob S (2022) Association Between Cardiac Radiation Exposure and the Risk of Arrhythmia in Breast Cancer Patients Treated With Radiotherapy: A Case–Control Study. Front. Oncol. 12:892882. doi: 10.3389/fonc.2022.892882

Background: Previous studies suggested that radiation therapy (RT) for breast cancer (BC) can induce cardiac arrhythmias and conduction disorders. However, the association with mean heart dose and specific cardiac substructures doses was less studied.

Materials and Methods: We conducted a nested case–control study based on French BC patients, enrolled in the European MEDIRAD-BRACE study (https://clinicaltrials.gov, Identifier: NCT03211442), who underwent three-dimensional conformal radiation therapy (3D-CRT) between 2009 and 2013 and were retrospectively followed until 2019. Cases were incident cases of cardiac arrhythmia. Controls without arrhythmia were selected with propensity-scored matching by age, duration of follow-up, chemotherapy, hypertension, and diabetes (ratio 1:4 or 5). Doses to the whole heart (WH), left and right atria (LA and RA), and left and right ventricles (LV and RV) were obtained after delineation with multi-atlas-based automatic segmentation.

Results: The study included 116 patients (21 cases and 95 controls). Mean age at RT was 64 ± 10 years, mean follow-up was 7.0 ± 1.3 years, and mean interval from RT to arrhythmia was 4.3 ± 2.1 years. None of the results on association between arrhythmia and cardiac doses reached statistical significance. However, the proportion of right-sided BC was higher among patients with arrhythmia than among controls (57% vs. 51%, OR = 1.18, $p = 0.73$). Neither mean WH dose, nor LV, RV, and LA doses were associated with an increased risk of arrhythmia (OR = 1.00, $p > 0.90$). In contrast, the RA dose was slightly

higher for cases compared to controls [interquartile range (0.61–1.46 Gy) vs. (0.49–1.31 Gy), $p = 0.44$], and a non-significant trend toward a potentially higher risk of arrhythmia with increasing RA dose was observed (OR = 1.19, $p = 0.60$). Subanalysis according to BC laterality showed that the association with RA dose was reinforced specifically for left-sided BC (OR = 1.76, $p = 0.75$), while for right-sided BC, the ratio of mean RA/WH doses may better predict arrhythmia (OR = 2.39, $p = 0.35$).

Conclusion: Despite non-significant results, our exploratory investigation on BC patients treated with RT is the first study to suggest that right-sided BC patients and the right atrium irradiation may require special attention regarding the risk of cardiac arrhythmia and conduction disorders. Further studies are needed to expand on this topic.

Keywords: breast cancer, radiation therapy, cardiotoxicity, cardiac arrhythmia, cardiac dosimetry

INTRODUCTION

Adjuvant radiation therapy (RT) after surgery is commonly used to treat localized breast cancer (BC) and generally results in significant improvement in tumor control and reduces the risk of cancer-related death several years after treatment (1, 2). However, BC survivors can develop a wide array of cardiotoxic complications related to cardiac radiation exposure, arising from a few months to many years after RT (3–5). Coronary artery disease is the most common manifestation of radiation-induced cardiovascular disease and also the most described in the literature (5). A relative increase of 7.4% in lifetime risk of coronary events for each Gy (Gray) of radiation to the heart has been demonstrated in women with previous BC having received radiation (6), reaching 16.5% for the first 9 years after RT (6, 7). Such complications are more commonly seen in patients with left-sided rather than right-sided BC as a larger portion of the heart, in particular the left anterior descending artery, is included in the radiation field (6, 8).

Among cardiac complications of thoracic RT, arrhythmia and conduction disorders are much less frequent than coronary artery disease [approximately 4%–5% (9)] and investigations on these complications were limited. Several case reports suggested a link between RT for BC and atrioventricular nodal bradycardia, and all levels of heart block, including complete heart block and sick sinus syndrome (10–12). Some cohort studies have shown that BC patients treated with RT had a higher risk of morbidity and mortality of cardiac arrhythmia than BC patients not treated with RT (13, 14). More recently, patients with BC who have undergone RT have been shown to have a 2.2-fold risk of conduction disorder requiring pacemaker implantation compared with the general population (15).

The question that remains is whether the risk of arrhythmia and conduction disorders, summarized under the general term "arrhythmia", is related to cardiac exposure due to RT. There are distinct etiologies for different types of radiotherapy-associated cardiotoxicity, and the dose–response relationship previously observed between the mean heart dose and the coronary complications cannot be directly applied to arrhythmias. Very few studies have evaluated whether the risk of arrhythmia increases with mean heart dose and specific cardiac substructure doses. In a study performed on lung cancer patients treated with RT between 1996 and 2009, arrhythmic events showed borderline significant associations with the whole heart dose and right atrium doses, but not with left ventricle or left atrium doses (16). However, cardiac radiation exposure and dose distributions are very different according to the type of cancer treated and further studies remain needed, particularly for BC patients who have undergone RT.

In order to specifically investigate the potential relationship between cardiac exposure and the risk of arrhythmia in BC patients treated with RT, we conducted a study to assess the association with whole heart and cardiac substructure doses including left and right ventricles, and left and right atria.

MATERIALS AND METHODS

Study Population

The nested case–control study was based on the French subgroup of left- and right-sided BC patients ($n = 347$) included in the multicenter MEDIRAD BRACE study further detailed elsewhere (ClinicalTrials.gov Identifier: NCT03211442).

The study population was composed of female patients, aged 40–75 years, who had undergone radiotherapy (3D-CRT) for a histologically proven diagnosis of BC (invasive and *in situ*) at Clinique Pasteur in Toulouse between January 2009 and December 2013.

After the surgical treatment of BC, all patients were treated with 3D-CRT with 6 and 25 MV photon beams by tangential fields, possibly including regional lymph node irradiation (internal mammary chain and supra-infraclavicular lymph nodes). The planning target volume dose was mostly 50 Gy delivered in 25 daily fractions of 2 Gy over 5 weeks or less frequently 32.5 Gy delivered in 5 daily fractions of 6.5 Gy. For most patients, 6 MV photons were used, except for a few cases of patients with big breast where 25 MV additional photons were used. An additional boost of 9–15 Gy could be applied to the tumor site using photon/electron beams with energies ranging from 6 MeV to 18 MeV. The treatment planning system (TPS)

used to perform dose calculations was Eclipse™ with the Analytical Anisotropic Algorithm (AAA v13.6) (Varian Medical System, Palo Alto, CA, USA). Each patient's RT was planned such that the dose distribution was optimized and normalized to the International Commission on Radiation Units and Measurements (ICRU) reference point of the breast and to achieve QUANTEC dose constraints to organs at risk including the heart (17), and deep-inspirational breath-hold (DIBH) technique was used for very few left-sided patients.

Patients' characteristics at baseline including comorbidities and history of cardiac arrhythmia (conduction disorders or arrhythmia) were extracted from medical records of the Clinique, completed with medical records of patients' general practitioners.

Patients with bilateral tumors, with distant metastasis at initial diagnosis, with previous RT before their initial BC treatment, with a history of cardiac arrhythmia, or without computed tomography for RT planning were excluded.

Follow-up data were retrospectively extracted from patients' medical records from their general practitioners from the date of RT through July 2019. Based on these medical records, we defined incident arrhythmia cases as any conduction disorders or arrhythmia events recorded by patients' general practitioners between the date of RT and July 2019.

To select controls corresponding to each arrhythmia case, propensity score matching with nearest-neighbor pairing was performed (18) based on factors known to potentially increase arrhythmia risk, including age at BC diagnosis, duration of follow-up (time from radiotherapy to the last observed follow-up time ≤ July 2019), the use of chemotherapy, and history of hypertension or diabetes. Laterality was not considered in propensity score matching in order to prevent overmatching between cases and controls regarding cardiac exposure. At least 4 or possibly 5 BC controls corresponding to each arrhythmia case were matched.

Radiation Dosimetry

For all patients, cardiac structure delineation was performed by UMCG using multi-atlas-based automatic segmentation (MABAS). The whole heart (WH) and its substructures, including the left and right ventricle (LV and RV), and the left and right atria (LA and RA), were recontoured using the MABAS tool of the heart developed in-house based on the atlas by Feng et al. (19) (Mirada RTx [version 1.6]; Mirada Medical, Oxford, United Kingdom) (20). The exact planned radiation dose was reconstructed from the delineated volumes, and dose-volume histograms were obtained for each patient. In the current analysis, mean doses (Dmean, in Gy) to the whole heart and cardiac substructures were considered to evaluate the dose–response relationship with the risk of arrhythmia.

Statistical Analysis

Conditional logistic regression, conditioned on the matching propensity score (including age, duration of follow-up chemotherapy, hypertension, and diabetes), was used to estimate the odds ratios of incident arrhythmia associated with clinical characteristics, BC laterality, and cardiac doses (for whole heart, left and right ventricles, and left and right atria). For cardiac doses, we analyzed Dmean as continuous variables, and evaluated the risk for higher doses (Dmean > 75th percentile) taking the group of patients with Dmean < 75th percentile as the reference group. Comparisons of doses according to BC laterality or case/control status were analyzed with paired Wilcoxon signed ranks tests. Spearman correlations were performed to evaluate the strength of the association between the whole heart dose and other cardiac substructure doses. Significance tests were two sided. A value of $p < 0.05$ was considered statistically significant. Statistical analysis was performed with the use of SAS Enterprise Guide version 4.3 and SAS version 9.4 (SAS Institute Inc, Cary, NC).

RESULTS

Patient Characteristics

The study included 116 patients: 21 incident arrhythmia cases and 95 controls without arrhythmia (5 matching controls were found for 11 cases and 4 matching controls were found for the 10 other cases). Mean age at radiotherapy was 64 ± 10 years and mean follow-up corresponding to the time from radiotherapy to the last observed follow-up time (≤ July 2019) was 7.0 ± 1.3 years. The mean interval from radiotherapy to arrhythmia was 4.3 ± 2.1 years. Matched characteristics were similar in cases and controls (**Table 1**). We observed higher frequencies of mastectomy, hormonal therapy, smoking status, hypercholesterolemia, or dyslipidemia in cases compared to controls, with corresponding OR ranging from 1.23 for dyslipidemia to 2.03 for hormonal treatment, but none of these covariates reached statistical significance in OR evaluations. Despite a slightly higher frequency of right-sided BC among cases compared to controls (57% vs. 51%), right laterality could not be established as a statistically significant risk factor of arrhythmia [OR = 1.18 (95% CI 0.47–3.08), $p = 0.73$].

Whole Heart and Cardiac Substructure Dosimetry

Median mean dose to the whole heart was 0.97 Gy, with higher doses for left-sided BC compared to right-sided BC (3.38 Gy vs. 0.59 Gy, $p < 0.0001$). Similar findings were observed for the left ventricle, right ventricle, and left atrium, with higher doses for left-sided BC (**Table 2**). In contrast, for the right atrium, doses were significantly higher for right-sided BC compared to left-sided BC (1.33 Gy vs. 0.50 Gy, $p < 0.0001$). The correlations of the whole heart dose with cardiac substructure doses were high for the left and right ventricles and the left atrium, independently from BC laterality (**Figure 1**). For the right atrium, the correlation with the whole heart dose showed a spurious negative association ($r = -0.28$) resulting from differences according to laterality of BC: the group of patients with a lower whole heart dose (corresponding to right-sided BC) could receive high right atrium doses whereas the group of patients with a high whole heart dose (corresponding to left-sided BC) received lower right atrium doses. Subanalysis of correlation between the whole heart and right atrium doses according to BC laterality showed a positive correlation for left-sided BC ($r = 0.75$) and right-sided BC ($r = 0.91$). In order

TABLE 1 | Clinical characteristics at initiation of radiotherapy for BC and relative risk of arrhythmia.

Characteristics	Cases (*n* = 21)	Controls (*n* = 95)	Odds Ratio (95% CI)	*p*-value
Matched characteristics				
Age at diagnosis, in years	66.14 ± 10.95	64.06 ± 10.25	NA	NA
Follow-up, in years	6.99 ± 1.53	6.97 ± 1.76	NA	NA
Chemotherapy, *N* (%)				
No	15 (71.43)	64 (67.37)	NA	NA
Yes	6 (28.57)	31 (32.63)		
Hypertension, *N* (%)				
No	8 (38.10)	40 (42.11)	NA	NA
Yes	13 (61.90)	55 (57.89)		
Diabetes, *N* (%)				
No	20 (95.24)	92 (96.84)	NA	NA
Yes	1 (4.76)	3 (3.16)		
Other characteristics				
Type surgery, *N* (%)				
Lumpectomy	18 (85.71)	87 (92.55)	1.00	0.37
Mastectomy	3 (14.29)	7 (7.45)	1.97 (0.45–8.70)	
Hormonal therapy, *N* (%)				
No	4 (19.05)	30 (31.58)	1.00	0.25
Yes	17 (80.95)	65 (68.42)	2.03 (0.61–6.78)	
Smoke, *N* (%)				
No	18 (85.71)	83 (87.37)	1.00	0.75
Yes	3 (14.29)	12 (12.63)	1.27 (0.29–5.67)	
Hypercholesterolemia, *N* (%)				
No	9 (42.86)	50 (52.63)	1.00	0.55
Yes	12 (57.14)	45 (47.37)	1.35 (0.51–3.58)	
Dyslipidemia, *N* (%)				
No	11 (52.38)	56 (58.95)	1.00	0.67
Yes	10 (47.62)	39 (41.05)	1.23 (0.47–3.23)	
Laterality of BC				
Left-sided BC, *N* (%)	9 (42.86)	47 (49.47)	1.00	
Right-sided BC, *N* (%)	12 (57.14)	48 (50.53)	1.18 (0.46–3.04)	0.73

95% CI: 95% Confidence Interval. "NA" for "Not Applicable".

TABLE 2 | Whole heart and cardiac substructure doses for all patients and according to BC laterality.

	All patients (*N* = 116) Median (interquartile range) Min–Max	Left-sided BC (*n* = 56) Median (interquartile range) Min–Max	Right-sided BC (*n* = 60) Median (interquartile range) Min–Max	*p*-value Left vs. Right
Dmean Whole Heart, Gy	0.97 (0.58–3.30) 0.0021–11.47	3.38 (1.56–4.80) 0.79–11.47	0.59 (0.46–0.71) 0.0021–1.48	<0.0001
Dmean Left Ventricle, Gy	0.41 (0.16–4.33) 0.0009–13.22	4.47 (1.99–6.61) 1.01–13.22	0.15 (0.11–0.21) 0.0009–0.77	<0.0001
Dmean Right Ventricle, Gy	0.95 (0.61–2.24) 0.0012–18.96	2.45 (1.25–4.49) 0.69–18.96	0.62 (0.44–0.78) 0.0012–1.59	<0.0001
Dmean Left Atrium, Gy	0.51 (0.39–0.76) 0.0028–3.69	0.70 (0.55–0.92) 0.22–3.69	0.41 (0.36–0.50) 0.0028–1.76	<0.0001
Dmean Right Atrium, Gy	1.00 (0.49–1.34) 0.0024–4.21	0.50 (0.34–0.60) 0.13–1.27	1.33 (1.14–1.64) 0.0024–4.21	<0.0001

Dmean, mean dose.

to consider the right atrium dose according to the whole heart dose, we analyzed the ratio Dmean RA/Dmean WH, which showed that the right atrium dose was 0.17 times lower than the whole heart dose for left-sided BC and 2.42 times higher than the whole heart dose for right-sided BC (**Table 3**).

Impact of Cardiac Doses on the Risk of Arrhythmia

Our study was limited in size, and none of the results presented here reached statistical significance. However, there are several findings. For the whole heart, left and right ventricles, and left atrium, lower doses were observed for cases compared to controls, without reaching statistical significance (**Figure 2**). However, for the right atrium, mean doses were slightly higher for cases than controls (cases: median = 1.04 Gy, interquartile range = 0.61–1.46; controls: median = 0.98 Gy, interquartile range 0.49–1.31; $p = 0.44$). As previously indicated, cases with arrhythmia were more likely to be right-sided BC than controls, without reaching statistical significance [OR = 1.18 (0.46–3.04)]. The frequency of patients receiving high doses (corresponding to

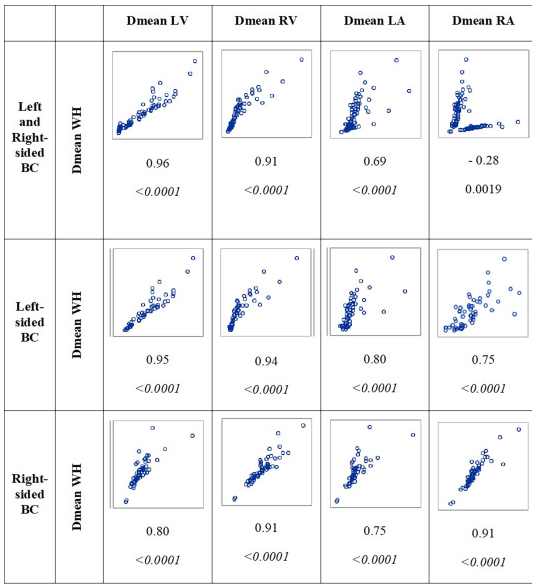

FIGURE 1 | Correlations between whole heart dose (Dmean WH) and cardiac substructure doses (left ventricle, right ventricle, left atrium, and right atrium) for all patients and according to BC laterality. *r* and the corresponding *p*-value are indicated below the graphs.

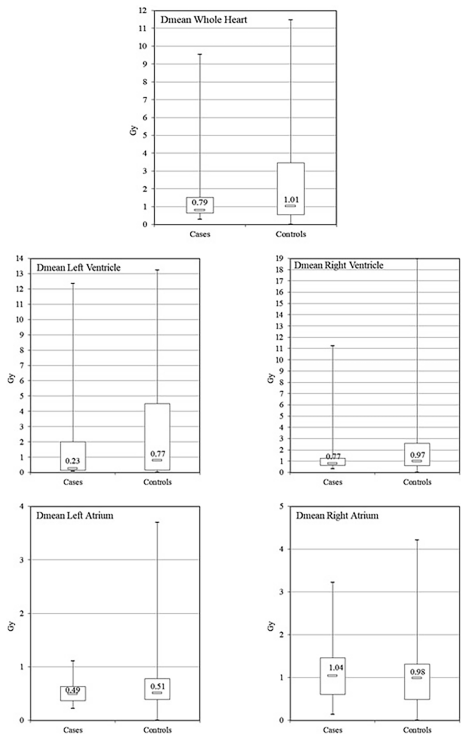

FIGURE 2 | Comparison of mean dose distribution for whole heart and cardiac substructures according to case or control status. [The central value of the box indicates the median, the borders of the box indicate the quartiles (25th and 75th), and the extremities indicate the minimum and maximum values.

TABLE 3 | Ratio Dmean RA/Dmean WH for all patients and according to BC laterality.

	RatioDmean RA/Dmean WH		
	Mean ± SD	**Median**	**Min–Max**
All patients	1.34 ± 1.17	1.75	0.07–4.06
Left-sided BC	0.17 ± 0.08	0.15	0.07–0.36
Right-sided BC	2.42 ± 0.42	2.36	1.14–4.05

doses > 75th percentile of dose distribution) to the whole heart, left and right ventricles, and left atrium (>3.30 Gy, 4.33 Gy, 2.24 Gy, and 0.76 Gy, respectively) was lower among cases than among controls (19% vs. 26%), yielding an OR < 1 but not statistically significant. Moreover, the risk of arrhythmia did not increase with increasing mean dose to the whole heart [OR = 1.00 (0.81–1.25), p = 0.98], and similar findings were observed for left and right ventricles and left atrium doses (OR = 1.00, 1.00, and 0.54, respectively) (**Table 4**). In contrast, the frequency of patients receiving high doses to the right atrium (>1.34 Gy) was higher among cases than among controls (33% vs. 22%), yielding an OR = 1.50 (0.58–3.88), p = 0.39, not statistically significant. A non-significant trend toward a potentially higher risk of arrhythmia with increasing mean dose to the right atrium was observed [OR = 1.19 (0.63–2.23), p = 0.60]. Subanalysis according to BC laterality (**Table 5**) showed that for left-sided BC, despite being non-significant, a potential association with the right atrium dose was observed (OR = 1.76, p = 0.75), as well as with the whole heart dose (OR = 1.28, p = 0.26). For right-sided BC, no direct association with RA dose alone was observed, but the ratio of mean doses RA/WH may have potential to predict arrhythmia (OR = 2.39, p = 0.35).

DISCUSSION

This exploratory study on the association between cardiac exposure and the risk of arrhythmia in BC patients treated with RT has several important findings. First, regarding the whole heart and the four cardiac chamber doses, the right atrium dose is the only structure presenting higher doses for right-sided BC compared to left-sided BC. Patients with arrhythmia were more likely to have right-sided BC than patients without arrhythmia. This could be explained by higher doses to the right atrium for right-sided BC. Second, an increased risk of arrhythmia was suggested with increasing dose to the right atrium, either directly, for patients with left-sided BC, or proportionally to the whole heart dose, for patients with right-sided BC. These results illustrate the potential relevance of right atrium exposure regarding the risk of arrhythmia.

Cardiac arrhythmias and bradycardia including conduction disorders are a broad category of potential complications of radiotherapy for BC as observed in previous studies. In a cohort of 746 BC patients, the cumulated incidence at 10 years of

TABLE 4 | Association between cardiac doses and risk of arrhythmia: continuous trend and values > 75th percentile of dose distribution.

Laterality or cardiac doses	% cases vs.% controls	Odds Ratio* (95% CI)	*p*-value
Right-sided BC [a]	57% vs. 51%	1.18 (0.46–3.04)	0.73
Whole Heart			
Dmean, in Gy	–	1.00 (0.81–1.25)	0.98
Dmean > 3.30 Gy [b]	19% vs. 26%	0.76 (0.23–2.49)	0.66
Left Ventricle			
Dmean, in Gy	–	1.00 (0.86–1.17)	0.98
Dmean > 4.33 Gy [c]	19% vs. 26%	0.77 (0.24–2.84)	0.65
Right Ventricle			
Dmean, in Gy	–	1.01 (0.86–1.19)	0.91
Dmean > 2.24 Gy [d]	19% vs. 26%	0.76 (0.23–2.55)	0.66
Left Atrium			
Dmean, in Gy	–	0.54 (0.14–2.08)	0.37
Dmean > 0.76 Gy [e]	19% vs. 26%	0.70 (0.22–2.24)	0.54
Right Atrium			
Dmean, in Gy	–	1.19 (0.63–2.23)	0.60
Dmean > 1.34 Gy [f]	33% vs. 22%	1.50 (0.58–3.88)	0.39

Odds ratios are unadjusted as none of the baseline characteristics in **Table 1 reached p-value < 0.20; CI: confidence interval. [a]Left-sided BC as reference category; [b]Dmean ≤ 3.30 Gy as reference category; [c]Dmean ≤ 4.33 Gy as reference category; [d]Dmean ≤ 2.24 Gy as reference category; [e]Dmean ≤ 0.76 Gy as reference category; [f]Dmean ≤ 1.34 Gy as reference category. BC, breast cancer; Dmean, mean dose; WH, whole heart; RA, right atrium.*

TABLE 5 | Subgroup (left-sided BC and right-sided BC) analysis of the association between risk of arrhythmia and whole heart and right atrium doses.

	Odds Ratio*(95% CI)	*p*-value
Left-sided BC		
Dmean WH, in Gy	1.28 (0.83–1.98)	0.26
Dmean RA, in Gy	1.76 (0.05–59.54)	0.75
Ratio Dmean RA/Dmean WH	0.90 (0.0–na)	0.99
Right-sided BC		
Dmean WH, in Gy	0.33 (0.02–5.20)	0.43
Dmean RA, in Gy	0.90 (0.30–2.25)	0.70
Ratio Dmean RA/Dmean WH	2.39 (0.38–14.90)	0.35

Odds ratios are unadjusted as none of the baseline characteristics in **Table 1 reached p-value < 0.20; CI: confidence interval; BC, breast cancer; Dmean, mean dose; WH, whole heart; RA, right atrium.*

arrhythmia/conduction disorder reached 4.8% in patients treated with RT versus 0% in patients not treated with RT (14). In this study, the definition of the event was broadly defined by arrhythmia/conduction disorder without additional information. In a French study based on a national healthcare database including a group of 2,973 BC patients treated with RT between 2008 and 2016 with a mean follow-up of 6 years, 28 cases of *de novo* pacemaker implantation were observed, which was 2.18 times higher than expected in the general population, whereas in the group not treated with RT, the number of pacemaker implantation was similar to the one expected in the general population (15). However, the relationship between the occurrence of these events and the level of cardiac radiation exposure has been sparsely studied.

There are distinct etiologies for different types of radiotherapy-associated cardiotoxicity, and the whole heart dose may not be the best predictor of all types of radiation-related heart disease (21). The occurrence of cardiac arrhythmias and conduction disorders after RT is usually associated with fibrosis, which might lead to alteration of conduction pathways with associated fibrosis of nodal structures (sinus and atrioventricular nodes) leading to rhythm changes (11). The tissue fibrosis induced by RT could be responsible for non-specific secondary cardiac lesions at the atrial, ventricular, and coronary levels, which are the basis for arrhythmias and bradycardia. However, the relatively low radiation doses and short follow-up in our study may not have caused significant cardiac radiation fibrosis. The sino-atrial node is located in the wall (epicardium) of the right atrium. The compact atrioventricular node is also located in the right atrium, close to the interatrial septum and the coronary sinus ostium. As a consequence, the right atrium dose may be a relevant proxy of these nodes' dosimetry and relevant for atrial arrhythmia and conduction disorders. In our study, we observed that right-sided irradiation was associated with a greater exposure to the right atrium, in contrast with other cardiac substructures, as previously observed (22). The analysis of the correlation between the whole heart dose and cardiac substructure doses showed strong correlation between the whole heart dose and left and right ventricle doses (left ventricle: $r = 0.95$ for left-sided BC and slightly lower $r = 0.80$ for right-sided BC; right ventricle: $r = 0.94$ for left-sided BC and 0.91 for right-sided BC). Similar findings were observed previously for whole heart and left ventricle doses with $r = 0.78$ for left-sided BC and lower $r = 0.55$ for right-sided BC (23). However, we found a moderate correlation between whole heart

and right atrium doses for left-sided BC patients (r = 0.75), illustrating that the mean heart dose may not be a good surrogate parameter of the right atrium dose, prompting a specific investigation of this cardiac substructure exposure regarding the risk of arrhythmia.

Some previous studies have investigated the relationship between doses to particular cardiac substructures and subsequent damage to those structures in BC patients, relating coronary artery doses to subsequent coronary artery stenosis (24) or left ventricle dose to subclinical left ventricular abnormalities (25, 26). In our study, the results suggested that the right atrium dose may be a better predictor of arrhythmia than the whole heart dose. For right-sided BC, the mean right atrium dose/mean heart dose ratio may be an interesting predictor of arrhythmia (OR = 2.39, p = 0.35). Such association with right atrium exposure would indeed be more relevant for atrial arrhythmias or conduction disorders more than ventricular arrhythmias, but this could not be checked in our data with no specific detail on arrhythmia/conduction disorders. Despite differences in the type of cancer treated and consequently level of cardiac exposure during cancer treatment, the association between right atrium dose and the risk of arrhythmia was previously presented in a cohort of 112 lung cancer patients treated with radiotherapy, followed on average for 9 years, among whom 12 arrhythmic events had been identified. Similarly to our study, no details on the type of arrhythmic event were provided, and this study, despite the fact that lung cancer RT led to higher radiation doses to the heart than in the current BC study, showed a relatively weak association with the whole heart dose (HR = 1.02, p = 0.054) and right atrium doses (HR = 1.02, p = 0.054), but not with the left ventricle or left atrium doses (16).

Studies that have analyzed the impact of cardiac dosimetry on potentially critical substructures for arrhythmias such as the sino-atrial node or the atrioventricular node are rare (27, 28). The prominent pacemaker role of the right atrium nodes should be kept in mind, and arrhythmia might be a long-term cardiac adverse event to consider specifically for right-sided BC patients. These nodal structures can be located, with some uncertainty, on the RT computed tomography and could therefore provide information on the association between cardiac exposure and the risk of atrial arrhythmias and conduction disorders (29).

We acknowledge that this study has some limitations. First, the size of the sample was small and the duration of follow-up was not very long, resulting in low statistical power, which could explain the absence of statistical significance in results. However, this study was exploratory, and its results encourage researchers to further analyze the risk of arrhythmia in larger studies. The definition of the arrhythmia event was based on medical records from BC patients' general practitioners. Despite the intensive work to collect data through general practitioners, we cannot be sure that the identification of incident cases of arrhythmia was complete. Consequently, some arrhythmia events among selected controls may be present. Such possible classification bias would nevertheless tend to dilute our results and not yield to spurious enhanced risk related to dose. Furthermore, no detail on the type of arrhythmia (atrial, ventricular, and conduction disorder) was provided, which may be of interest for better knowledge on the occurrence of these events. Whole heart and cardiac substructure dosimetry was based on MABAS. Such methodology was previously validated, showing reliability and efficiency for contouring of cardiac substructures and obtaining cardiac dose parameters with accuracies at least similar to interobserver delineation variation (20). However, such methodology may, in some patients, be less precise than manual segmentation, involving possible inaccuracies in the doses and resulting in "noise" in the evaluation of dose–response relationship. None of the potential risk factors of arrhythmia, including hormonal therapy for example (OR = 2.03, p = 0.25), reached statistical significance, which may mainly reflect an under power of our study as stated above. Hormonal therapy combined with radiotherapy may increase the risk of arrhythmia (30), but this could not be evaluated in our study. Cardiac radiation doses in BC therapy are low compared to certain other thoracic cancer therapies that were previously considered in previous studies such as lung cancer, resulting in lower dose ranges and issues that highlight the potential RT-associated risk. Another limitation is the use of old techniques of irradiation in our study. DIBH can substantially decrease cardiac exposure in patients (22), and intensity-modulated radiotherapy with helical tomotherapy has shown a very low rate of cardiac complications (31).

Further studies remain needed in order to enhance knowledge on the risk of arrhythmia and conduction disorder after RT for BC and refine the potential relationship between the risk of arrhythmia and exposure to cardiac substructures and, *in fine*, identify "high risk" patient profiles. These studies will require a larger number of patients, inclusion of patients treated with modern techniques of RT (DIBH, IMRT, proton therapy, and other adapted techniques), detailed cardiac substructures, and nodal dosimetry. Collecting information on the kind of arrhythmia (atrial or ventricular arrhythmia, conduction disorder) is also an important perspective as there may be differences in radiation sensitivity of various cardiac tissues (ventricular and atrial myocardium, conductive tissue, nodes, etc.).

CONCLUSION

Our exploratory study on the risk of cardiac arrhythmia in BC patients treated with RT suggested that right-sided BC patients may require particular attention and the dose to the right atrium may be a more relevant dosimetry parameter than the whole heart or other cardiac substructure doses regarding the risk of arrhythmia. Such findings may be related to the location of the sino-atrial node (pacemaker cells) and conductive tissue in the right atrium and prompt researchers to further investigate more specifically these structures' dosimetry.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, on reasonable request.

ETHICS STATEMENT

The study was subject to a declaration of compliance with a reference methodology concerning research in the field of health (MR 03) from the French Commission Informatique et Liberté - CNIL, (ref 2103119, September 27, 2017). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation was carried out by ME, ML, DS, JAL, AC, and SJ. Data collection and analysis were performed by MYE, ML, DS, GJ, JC, MOB, AC, and SJ. All authors participated in the writing of the manuscript. All authors read and approved the final manuscript.

FUNDING

This work was funded by H2020 Euratom research and training program 2014–2018 under grant agreement No. 755523 in the frame of the MEDIRAD project.

ACKNOWLEDGMENTS

The authors thank Fanny Paris, clinical research associate, for assistance in the collection of French patients' data.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer MB is currently organizing a Research Topic with the author VM.

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REVIEW
published: 19 July 2022
doi: 10.3389/fonc.2022.934369

Role of Real-World Data in Assessing Cardiac Toxicity After Lung Cancer Radiotherapy

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Radiation-induced heart disease (RIHD) is a recent concern in patients with lung cancer after being treated with radiotherapy. Most of information we have in the field of cardiac toxicity comes from studies utilizing real-world data (RWD) as randomized controlled trials (RCTs) are generally not practical in this field. This article is a narrative review of the literature using RWD to study RIHD in patients with lung cancer following radiotherapy, summarizing heart dosimetric factors associated with outcome, strength, and limitations of the RWD studies, and how RWD can be used to assess a change to cardiac dose constraints.

Keywords: real-world data, lung cancer, cardiac toxicity, radiation induced heart disease, heart dose constraints

OPEN ACCESS

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Specialty section:
This article was submitted to Radiation Oncology, a section of the journal Frontiers in Oncology

Received: *02 May 2022*
Accepted: *13 June 2022*
Published: *19 July 2022*

Citation:
Abravan A, Price G, Banfill K, Marchant T, Craddock M, Wood J, Aznar MC, McWilliam A, van Herk M and Faivre-Finn C (2022) Role of Real-World Data in Assessing Cardiac Toxicity After Lung Cancer Radiotherapy. Front. Oncol. 12:934369. doi: 10.3389/fonc.2022.934369

INTRODUCTION

Radiotherapy is the recommended treatment for approximately 50% of patients with cancer (1). Despite advances in radiotherapy techniques, some degree of radiation-induced toxicity remains inevitable. There is increasing evidence that cardiac toxicity is a concern in patients with lung cancer receiving radiotherapy and can occur earlier than previously thought. The impact of radiotherapy dose to the heart or sub-regions of the heart in patients with lung cancer receiving radiotherapy on overall survival (2–12), non-cancer deaths (13, 14), and incidence of cardiac events/deaths was recently demonstrated (2, 7, 8, 10, 15–20). However few studies have also incorporated the effect of baseline cardiac comorbidities or polypharmacy on radiation-induced heart disease (RIHD) and treatment outcome (8, 10, 15–20).

Randomized controlled trials (RCTs) are the gold standard method of providing evidence relating to efficacy and tolerability of treatment in the modern healthcare system (21). There are however many clinical scenarios, particularly in the radiotherapy setting, where there is no data available from RCTs and/or conducting RCTs is challenging, and therefore there is no clinical consensus on standard of care treatment. For example, older, frailer patients and those who present with higher level of comorbidities at diagnosis are well-known to be under-represented in RCTs (22), and as such, evidence to support decision making in these patient populations is often limited (23–25). Moreover, as radiotherapy advances rapidly, with much of its modification occurring through successive incremental technical developments rather than transformative step-changes,

the impacts of such changes are challenging to test using classical RCTs. Furthermore, there is often a learning effect associated with new technologies, and hence a risk that results can quickly become outdated. Finally, there are common situations where trials would be difficult to design due to lack of clinical equipoise. For example when introducing image-guided radiotherapy (IGRT), many believed that imaging-based treatment would likely be superior to non-imaging-based treatment with regard to outcomes such as local control (26).

An alternative to RCTs is to provide evidence from the real-world setting that has the advantage of being more inclusive. Food and Drug Administration (FDA) defined Real-World Data (RWD) as "the data relating to patient health status and/or the delivery of health care routinely collected from a variety of sources" (27). Both the FDA and National Institute for Health and Care Excellence (NICE) are recognizing the importance of using routine data to evaluate how interventions tested in highly selected cohorts translate to the general population (28–30). In the field of cardiac toxicity, much of the information we have available comes from studies that used RWD. *Post-hoc* studies of cardiac toxicity from clinical trials of thoracic radiotherapy and retrospective studies of heart dosimetry and outcome can indeed be hypothesis generating e.g., on the interplay between baseline comorbidities and RIHD and the impact of cardiac dose on patient outcomes (2, 4–6, 16, 17, 31–33).

The aim of this article is to provide a narrative review of RWD studies in the field of RIHD in patients with lung cancer. By RWD studies, we mean studies that include data from patients that are not recruited to interventional experimental studies with specific inclusion/exclusion criteria. We summarize the existing literature derived from RWD, including data on heart dosimetric factors linked to outcome. Finally, we recap the strength and limitations of the RWD studies in this setting and describe how RWD can be used to evaluate a change to cardiac dose constraints.

REAL-WORLD DATA IN THE CONTEXT OF RADIATION INDUCED HEART DISEASE

Clinical Context

Current cardiac dose constraints are mainly based on the Quantitative Analysis of Normal Tissue Effects in the Clinic (QUANTEC) and are mainly derived from radiotherapy in patients with esophageal cancer and lymphoma studies (34). In contrast to QUANTEC recommendations that mean heart dose should be kept below 15 Gy, Darby et al. presented a linear risk, no threshold model for major adverse cardiac events post-RT in a retrospective RWD case-control study that included >2000 individuals with breast cancer (30). In patients with lung cancer, survival remains poor; compared to patients with breast cancer, patients with lung cancer are typically older and have multiple comorbidities (35). The poor survival of patients with lung cancer taken together with the belief that RIHD has a long latency period based on data from the field of breast cancer and lymphoma (36, 37), have led to the underestimation of the risk of cardiac toxicity related to thoracic radiotherapy in patients with lung cancer. Moreover, higher cardiac dose exposure in patients with lung cancer may result in earlier onset of RIHD.

RTOG 0617 was the landmark clinical trial that kick-started worldwide awareness and interest in the field of RIHD in this setting. This RCT comparted a standard dose of thoracic radiotherapy (60 Gy in 30 fractions) to a higher dose (74 Gy in 37 fractions) delivered concurrently with chemotherapy +/- cetuximab (38). High-dose radiotherapy was associated with a higher risk of mortality, and multivariable models demonstrated that heart dose is an important prognostic factor for all-cause mortality (2). However, specific heart toxicity endpoints were not recorded in the trial, therefore the association of dose with RIHD or cardiac death could not be assessed. Wang et al. subsequently presented an analysis of pooled data from six lung cancer dose-escalation trials with endpoints for symptomatic cardiac death (15). In competing risk-adjusted cumulative incidence curves, for cardiac death, the impact of higher mean doses to the heart was shown. Patients receiving greater than 20 Gy mean heart dose were more than twice as likely to experience death due to a cardiac cause than patients with a mean heart dose of 10 Gy or less.

These *post-hoc* analyses of RCT data stimulated interest in the field of RIHD in lung cancer and researchers have since sought to supplement this evidence using RWD. Following the publication of RTOG 0617, it has been recognized that the latency time for RIHD in patients with lung cancer is much shorter than other thoracic cancers including patients with lymphomas and breast cancers who typically develop RIHD at least 5 years after radiotherapy (37, 39–42).

There is also an appreciation that the physiology of RIHD is complex. The heart consists of several connected anatomical sub-structures, each of which could have an associated radiotherapy dose response to radiation. Identifying the structures with the strongest association with RIHD is challenging due to the proximity of these sub-structures, meaning that the radiation dose between neighboring regions will be highly correlated. Despite these challenges, studies based on RWD have identified dose to sub-regions of the heart more strongly associated with patient outcomes. McWilliam et al., using RWD and a voxel-based data mining approach, reported radiation dose to the base of the heart had the greatest impact on survival in patients with lung cancer treated with radical radiotherapy (3). This region was further validated in patients with stage I non-small cell lung cancer (NSCLC) treated with stereotactic body radiation therapy (SBRT). Stam et al. analyzed the dose to cardiac sub-structures using a template anatomy, identifying the superior vena cava and left atrium as most strongly associated with non-cancer death in patients with early-stage NSCLC receiving SBRT (13). Similarly to Darby et al's study in patients with breast cancer (36), in a RWD nested case-control study, Abravan et al. showed a linear relationship between mean dose to a region located at the base of the heart and cardiac-related deaths in patients with lung cancer treated with radical radiotherapy (43). No threshold has been identified, however, and whether such a relationship is linear or threshold

based still remains to be understood. **Table 1** shows the results of studies utilizing RWD to investigate associations between heart dosimetric parameters and outcome in patients with lung cancer. Of note, most studies in lung cancer investigate the link between outcomes and planned dose (as opposed to delivered dose). However, the set-up uncertainties and anatomical motion impacts the dose received by the heart and heart sub-structures, and thereby the risk of RIHD (44).

A complementary study from Manchester, again using RWD, investigated the impact of residual set-up errors on patient outcomes. For each fraction of radiotherapy, the patients' positioning is checked with an on-board cone-beam CT scan. Any positional differences in the tumor can be corrected; however, these corrections can result in shifting the radiation dose towards or away from the heart. Over the full treatment course, this may result in an individual patient receiving a higher or lower dose to the heart than planned. In patients with stage III NSCLC, when the dose was moved in the direction of the heart (likely increasing the heart dose), patients had worse overall survival in multivariable analysis (45). The same effect was seen in patients with early-stage NSCLC treated with SBRT (46). Further analysis of the dataset of patients with stage III NSCLC investigated the dose differences due to these positional changes and identified a region in the base of the heart where the changed dose was most strongly associated with early mortality (47). Importantly, variation in residual set-up errors can be considered as random and compared to a natural experiment with no obvious correlation with other clinical or patient characteristics, allowing a causal relationship between the dose response to the base of the heart and risk of death to be inferred.

In addition to the studies in the field of lung cancer, a number of RWD studies have been reported on the impact of dose to heart or heart sub-structures on risk of cardiac events in patients with other thoracic tumors. For example, in patients with esophageal cancer, despite high level of competing risk, association between heart dose and cardiac events (48, 49), key coronary substructures (namely left anterior descending coronary artery [LAD]) dose and incidence of major coronary events (50) have been reported. In patients with breast cancer, association between mean heart dose and major coronary events (36), left ventricle dose and cardiac events (51), and LAD dose and increased requirement for coronary intervention in mid LAD (52) have been reported. However, it should be noted that heart exposure from tangential fields during breast radiotherapy only affects a small section of the heart compared to the exposure observed during thoracic radiotherapy for lung cancer (where one or more beams traverse the heart). Therefore, difference in dose distribution within the heart, baseline comorbidities, and age at diagnosis may partly explain why RIHD is an acute event in patients with lung cancer as opposed to a late event in patients with breast cancer.

Baseline Cardiac Conditions

Identification of the burden and severity of cardiac comorbidities is important to personalize cardiac sparing in patients with lung cancer treated with thoracic radiotherapy. It has been established that comorbidities are an important predictor of early mortality (53). Indeed, about 75% of patients with lung cancer have known comorbidities at diagnosis with the most common being cardiovascular disease, chronic obstructive pulmonary disease, and diabetes (54–57). An area of interest in the field of cardiac toxicity in lung cancer is the impact of pre-existing cardiac condition on the risk of RIHD, given that a quarter of patients with lung cancer will present with a cardiovascular disease at diagnosis (55, 58, 59).

In a retrospective cohort of 1155 patients with lung cancer, Tammemagi et al. identified multiple comorbidities in two-thirds of patients, including 18 comorbidities that demonstrated stronger associations with early mortality than age, gender, or smoking (53). A United States National Cancer Institute Surveillance, Epidemiology and End Results (SEER) database RWD study of patients aged over 65 years with small cell lung cancer (SCLC) found that patients who had a cardiac event (acute myocardial infarction, cardiomyopathy, arrhythmia, heart failure or pericarditis) in the 12 months prior to treatment had an increased incidence of cardiac events following chemoradiotherapy (60). The rate of cardiac events was 55.4% in the year following radiotherapy in patients who had a previous cardiac event compared to 28% in those who had not had a previous cardiac event. A similar study in patients with NSCLC found an increased mortality in patients with known cardiac comorbidities following thoracic radiotherapy (59). Even when patients with cardiac comorbidities were excluded from the analysis, there was still an increase in cardiac events following radiotherapy in patients with multiple non-cardiovascular comorbidities (61).

Wang et al. paired World Health Organization/International Society of Hypertension (WHO/ISH) risk score with dosimetric parameters on multivariable analysis and found that patients with a high 10-year risk of a cardiovascular event had a significantly higher rate of cardiac events after radiotherapy (15). The WHO/ISH risk prediction charts not only indicate the risk of ischemic heart disease but also stroke and are only applicable to patients who have not yet had a cardiovascular event. Therefore their use is limited in a population of patients with lung cancer, over 25% of whom will have a history of a cardiovascular event (62). To overcome this issue, Dess et al. used the Framingham risk score, which predicted a patient's risk of myocardial infarction or death from ischemic heart disease in a cohort of 71 patients with lung cancer and without pre-existing cardiac disease treated with dose-escalated radiotherapy (16, 63). This *post-hoc* analysis did not find any correlation between Framingham risk score and ≥ grade 3 cardiac event.

In a retrospective study of 748 patients who had radiotherapy for stage II-III NSCLC, Atkins et al. showed not only that patients with cardiac comorbidities had an increased rate of major adverse cardiac events following treatment compared with those without cardiac comorbidities, but also that mean heart dose ≥ 10Gy was associated with increased incidence of major adverse cardiac events (cardiac death, unstable angina, and myocardial infarction) in patients without a history of ischemic heart disease (8). They further reported that mean heart dose is

TABLE 1 | Summarizing RWD studies suggesting associations between heart dosimetric factors and outcome of patients with lung cancer in multivariable models.

Authors, year	Patient stage, population (n)	Study Institution	Correlation between heart dosimetric factors, other heart related factors and outcome in multivariable analysis
Speirs et al, 2016 (2)	Stage II-III NSCLC (416)	Single institution, Siteman Cancer Center/ Barnes Jewish Hospital	Heart V_{50} associated with OS and cardiac toxicity
Mcwilliam et al, 2017 (3)	Stage I-IV Lung cancer (1101)	Single institution, The Christie	Mean dose to the identified region located in the base of the heart associated with OS
Stam et al, 2017 (13)	Stage I-II NSCLC (803)	Multi-institutional	Maximum dose on the left atrium and dose to 90% of the superior vena cava associated with non-cancer death
Stam et al, 2017 (4)	Stage II-III NSCLC (469)	Single institution, NKI	Heart V_2 associated with OS
Wang et al, 2017 (15)	Stage III NSCLC (112)	Multi-institutional	MHD, heart V_5, heart V_{30}, and left ventricle V_5 associated with CE in patients with IHD or high WHO/ISH risk scores. MHD ≥ 20 Gy higher rate of CE No association between OS and heart dose
Dess et al, 2017 (16)	Stage II-III NSCLC (125)	Multi-institutional	MHD and PCD associated with higher CE
Vivekanandan et al, 2017 (5)	Stage IIB-III NSCLC (78)	University of Oxford	ECG changes at 6 month and left atrium dose > 64 Gy associated with OS
Ning et al, 2017 (17)	Stage I-IV NSCLC (201)	Single institution, MD Anderson Cancer Center	Heart V35 >10% and PCD associated with PCE
Chun et al, 2017 (6)	Stage III NSCLC (482)	Multi-institutional	Heart V40 associated with OS
Yegya-Raman et al, 2018 (18)	Stage II-IV NSCLC (140)	Single institution, Rutgers Cancer Institute	MHD and baseline coronary artery disease associated with symptomatic CE
Wong et al, 2018 (14)	Stage I-II NSCLC (189)	Single institution, Princess Margaret Cancer Centre	Max dose (per 100 Gy) to left and right ventricle associated with non-cancer deaths
Xue et al, 2019 (7)	Stage I-III NSCLC (94)	Multi-institutional	MHD, hart V5, V55, pericardial mean dose, V5, V30, and V55 associated with PCE Pericardial V30 and V55 associated with OS
Atkins et al, 2019 (8)	Stage II-IIIB NSCLC (748)	Single institution, Dana-Farber Cancer Institute/ Brigham and Women's Hospital	MHD (≥10 Gy) associated with MACE and OS
Mcwilliam et al, 2020 (9)	Stage I-IV Lung cancer (978)	Single institution, The Christie	Max dose to the combined cardiac region including right atrium, right coronary artery, and ascending aorta associated with OS
Abravan et al, 2020 (19)	Stage II-III Lung cancer (1243)	Single institution, The Christie	Mean dose to the identified region overlapping with right atrium (≥10 Gy) associates with cardiac related death in patients without PCD
Atkins et al, 2021 (10)	Stage II-III NSCLC (701)	Single institution, Dana-Farber Cancer Institute/ Brigham and Women's Hospital	LAD coronary artery V15≥ 10% associated with MACE and OS, particularly in patients without CHD. Left ventricle V15 ≥ 1% associated with MACE in patients with CHD.
Shepherd et al, 2021 (11)	Stage I-III NSCLC (285)	Single institution, MSKCC	Heart V8 associated with OS
Abravan et al, 2021 (20)	Stage II-III Lung cancer (1218)	Single institution, The Christie	Mean dose to LAD associated with cardiac hospital admission and cardiac related death in patients without diagnosed PCD
Abravan et al, 2022 (43)	Stage II-III Lung cancer (2488)	Single institution, The Christie	Mean dose to cardiac avoidance region (superior vena cava, right atrium, aortic root, and proximal segments of the coronary arteries) linearly associated with the increase in the risk of cardiac related death

NSCLC, non-small cell lung cancer; OS, overall survival; MHD, mean heart dose; IHD, ischaemic heart disease; WHO/ISH, world health organization/international society of hypertension; CE, cardiac events; ECG, electrocardiogram; PCD, pre-existing cardiac disease; PCE, pericardial effusion; MACE, major adverse cardiac events; LAD, left anterior descending coronaryartery; CHD, chronic heart disease.

not a suitable surrogate for LAD dose and the risk of major adverse cardiac events in the sub analysis of the same lung cancer cohort (10).

By having access to cause of death from Public Health England data, and hospital admissions from Hospital Episode Statistics, and utilizing voxel-based data mining, Abravan et al. investigated how radiotherapy dose in the thorax relates to cardiac-related death taking into account patient pre-existing cardiac conditions, using RWD from 1243 patients with lung cancer (19). Fine and Gray competing risk regression for cardiac-related death, with other causes of death as a competing risk, showed an increase in the risk of cardiac-related death in patients with pre-existing cardiac disease. Voxel-based data mining identified a region overlapping with the right atrium where dose was significantly higher in those patients who died due to a cardiac cause. Multivariable analysis suggested that radiotherapy dose to this region has the highest impact on cardiac-related death only in those patients without diagnosed cardiac conditions prior to treatment. In a further study, Abravan et al. studied the risk of cardiac hospital admission after radiotherapy and dose delivered to cardiac sub-structures in 1218 patients with lung cancer with no known pre-existing cardiac disease (20). Multivariable analyses showed that mean LAD dose correlates with both cardiac admission post-RT and cardiac-related death. Cardiac admission post-RT also correlates with cardiac-related death in the model including mean LAD dose. It is suggested that significance of LAD dose alongside cardiac admission in predicting cardiac-related death may point to undiagnosed cardiac disease in this population.

Calcifications are one established predictor for cardiovasular events (64–68) and are directly measurable from the CT scan acquired for planning a patient's treatment. Utilizing RWD, Abravan et al. observed an association between the volume of calcifications found on the planning 4DCT scan and cardiac comorbidity scores obtained from Adult Co-Morbidity Evaluation (ACE-27) in 334 patients with lung cancer treated with SBRT (69). Multivariable models showed that the volume of calcification is an independent predictor of patient survival. Furthermore, for 428 patients, a deep-learning model was applied to identify calcifications from planning CT scans and stratify into low- and elevated-risk groups. Patients in the high-risk group were found to have an increased risk of all-cause mortality in the multivariable model (70).

Other Toxicities Related to Heart Radiotherapy Dose: Lymphopenia

Other toxicities can also result from heart irradiation and further affect the outcome of patients with lung cancer. Incidence of lymphopenia (a drop in lymphocyte counts), has for example been reported following thorax irradiation. Severe lymphopenia has been shown to be associated with worse outcome in patients with lung cancer who received radiotherapy as part of their cancer treatment (71, 72).

Few RWD studies have addressed the effect of heart irradiation on lymphopenia and outcome. Ladbury et al. reported that higher radiotherapy dose to the "host immune system," defined as a function of mean heart dose, mean lung dose, mean body dose, and number of fractions, was associated with overall survival in 117 patients with stage III NSCLC (72). Abravan et al. utilized a large cohort (>900) of patients with lung cancer receiving curative-intent radiotherapy and studied which organs are responsible for severe lymphopenia during radiotherapy when irradiated. Using voxel-based data mining, results showed an association between thoracic vertebrae V20, mean lung dose, mean heart dose and grade 3 or higher lymphopenia (71). Authors further showed that lymphopenia is an independent predictor for OS in both SCLC and NSCLC. Local irradiation to heart and lung affects circulating lymphocytes in the blood pool, which may explain one important mechanism of lymphopenia. Another study by Zhao et al. showed worse OS for 76 early-stage patients with lung cancer who developed grade 2 or higher lymphopenia after SBRT. A negative association between heart V5 and total lymphocyte count after SBRT was further indicated (73).

Evidence is emerging that both cardiac toxicity and lymphopenia are associated with cardiac irradiation, and further work is required to elucidate the relationship between toxic heart dose, lymphopenia, and patient outcome.

DISCUSSION

What are the Strengths and Limitations of Real-World Data?

RCTs have the advantage of ensuring high internal validity in a way that observed effects are the result of the tested intervention. They provide high quality data but often they require additional patient procedures incurring greater expense or burden for patients. The downside of RCTs is that they lack generalizability. RCTs can be subjected to selection bias and hence may not accurately represent the patient population of interest (74, 75). Moreover, RCTs are often expensive and there are situations where they can be impractical, such as evaluation of technological advances in radiotherapy (26). In the field of radiotherapy-induced cardiac toxicity, given the accumulating evidence on the impact of dose to specific anatomical regions of the heart, it is becoming increasingly difficult to argue equipoise for the evaluation of new dose limits through classical RCTs. In such situations, the application of RWD can provide an alternative to conventional RCT evidence. Whereas much of the RWD evidence discussed above comes from retrospective observational studies, RWD is not only synonymous with this approach but can also be used prospectively to study the impact of new interventions in pragmatic trial designs (76).

It is however important to acknowledge that RWD studies have known limitations, primarily the risks of bias introduced by missing or incorrectly recorded data, and the inherent risk of unmeasured confounding in non-randomized datasets. In RIHD studies for example, target volume and location may influence not only the dose to the heart but also clinical outcome (survival) which can lead to false association in real-world research and may affect the validity of evaluations of interventions (77). In

addition, dose exposure to one sub-structure in the heart is often co-linear with dose to another nearby sub-structure, such that the selection of which sub-structure or dose threshold is responsible for damage is usually done on statistical considerations, which is unlikely to reflect the underlying biology.

How to Achieve High Quality Real-World Data Research?

There are concrete steps that can be taken to improve the quality of the RWD required to study RIHD. For example, heart or heart sub-structure segmentations are essential to successfully derive high-quality and meaningful evidence to inform decisions in the clinic. However, retrospective contouring of structures or sub-structures of interest is not realistic. The manual contouring of heart sub-structures on routine radiotherapy planning CTs is a particularly challenging task as respiratory motion, cardiac motion, as well as the varying extent of co-morbidities can impair visualization and result in large inter-observer variation particularly of small structures, such as the valves or coronary arteries (78). Even when sub-structures have been prospectively contoured, important variations can be seen due to the different guidelines available, or different interpretation of existing guidelines (79). For example, Thor et al. (80) have analyzed the heart doses reported in the RTOG 0617 clinical trial and demonstrated that inconsistencies in delineation led to a significant underestimation of cardiac exposure. They concluded that auto-contouring (e.g., using deep-learning to segment the whole heart) could increase the quality of clinical trials and the reliability of dose-toxicity associations explored in secondary analyses.

Several groups have developed auto-segmentation tools, using either atlas-based (81, 82) or machine-learning approaches (83) to address this issue. The performance of these auto-contouring solutions continues to improve but is affected by uncertainties in the manual contours used for training/validation. Another avenue that some authors have pursued is to use motion compensation to improve the quality of planning CTs and reduce contour uncertainties. Even though the motion of heart sub-structures due to heartbeat is reportedly small (typically <5 mm) (84), the heart can move 5-20 mm due to respiration. With the adoption of 4DCT worldwide, the use of different reconstruction techniques may add uncertainty to heart sub-structure segmentation. A potential solution to mitigate this issue is the use of motion compensated (mid-position) reconstructions which have been shown to reduce inter- and intra-observer contouring variations for organs at risk in patients with lung cancer (85). Moreover, Abravan et al. evaluated cardiac calcification detection in different phases of the respiratory cycle and found better detection in the extreme position of the respiratory cycle (69).

Novel methodologies can be used to generate evidence about the effectiveness of new treatments such as heart-sparing radiotherapy where RCT data will not exist. The robustness of biases can be assessed by employing probabilistic bias analysis, an approach that systematically assesses the extent of potential confounders (86). Utilizing new approaches such as causal inference, in which expert assumptions about causal mechanisms of outcomes are directly incorporated into statistical models using observational data, may further help with minimizing the effect of confounding. For example, the study discussed previously in which positional set-up errors are used to infer changes in heart dose, uses an instrument variable approach that can be compared to a natural randomized experiment (45). Such studies increase confidence that the observed differences in survival are caused by the differences in heart dose and are not merely associations with different underlying causes.

How Results From Real-World Data Could Be Utilized in the Clinic?

The ultimate aim of RWD studies in the field of RIHD is to introduce and apply a dose limit to a defined anatomical area of the heart as part of the treatment planning process. If the evidence on sparing of anatomical regions of the heart is equivocal, equipoise can be argued and randomized designs such as pragmatic point-of-care or simple trial could be used (87). These approaches are designed to evaluate the effectiveness of interventions as an embedded part of routine practice, and are intermediate between RCTs and quasi-experimental studies. They aim to preserve a high degree of internal validity while reducing some of the disadvantages of conventional RCTs. However, as argued above, evidence in the field of radiotherapy-induced cardiac toxicity for patients with lung cancer is accumulating and it is becoming more difficult to argue equipoise. In this context, non-randomized quasi-experimental designs could instead be used in which specific outcome measures before and after a new clinical intervention is implemented are compared (76).

Use of Rapid Learning as a Methodology to Assess the Impact of Heart Dose on Survival

Recent RWD studies (3, 9, 19, 20), have shown that incidental dose to the base of the heart increases the risk of early mortality in patients with lung cancer. A cardiac avoidance region was defined based on our previous studies encompassing structures located at the base of the heart including superior vena cava, right atrium, aortic root, and proximal segments of the coronary arteries. It is hypothesized that the cardiac toxicity is a result of damage done to the conduction system and the coronary arteries through inflammation, fibrosis, or ischemia. The RAPID-RT programme recently funded by the UK National Institute for Health Research (NIHR) is a large-scale research programme which will evaluate a change in the radiotherapy protocol to spare a cardiac-avoidance region in patients with stage II-III lung cancer treated with curative-intent radiotherapy at The Christie NHS Foundation Trust in Manchester, UK (27). A summary of the evidence that has fed into this programme of research is summarized in **Figure 1**. This change in the radiotherapy protocol is expected to increase patients' short-term survival by 10-20%. Nevertheless, changing treatment delivery to spare the heart avoidance region without compromising tumor coverage

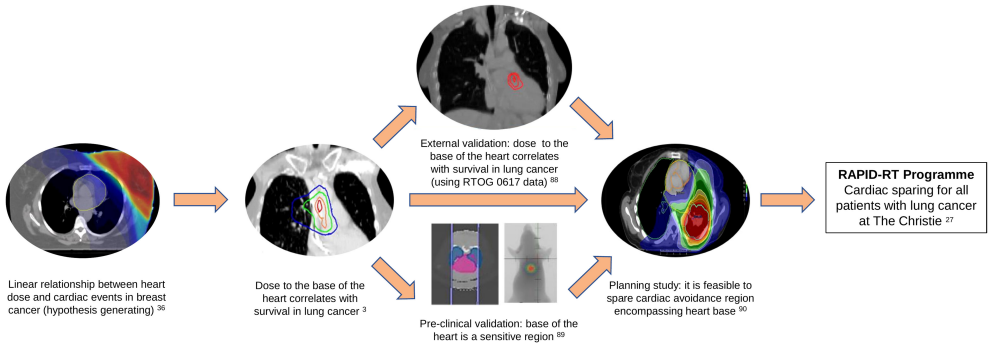

FIGURE 1 | Evidence leading to the development of the RAPID-RT programme of research. In 2013, Darby et al. reported a linear relationship between mean heart dose and major coronary events in patients with breast cancer treated with radiotherapy (36). Mcwilliam et al, in 2017, showed there is a strong correlation between dose received by the base of the heart and overall survival in patients with lung cancer (3). These findings were externally validated using data from RTOG 0617 trial (88). A pre-clinical study further validated that the base of the heart is a sensitive region (reverse translation) (89). A feasibility planning study demonstrated that it is possible to spare cardiac avoidance region (located at the base of the heart) on previous Manchester studies (90). The RAPID-RT programme, funded by the National institute for health research (NIHR), will evaluate the impact of the introduction of a dose limit to the cardiac avoidance region in all patients with stage II-III lung cancer treated with curative-intent radiotherapy at The Christie NHS Foundation Trust in Manchester, UK (27).

may increase the dose to other organs at risk nearby, primarily the lungs, which in return may increase the risk of other toxicities, such as radiation pneumonitis (27). The programme will use a quasi-experimental interrupted time series design, with multiple cycles of learning, to assess the impact of the introduction of a dose limit to the cardiac avoidance region on survival and other toxicities using RWD. Related studies will assess the quality of the evidence derived from the rapid-learning methodology and how either it, or the methodological RWD approach, can be used to contribute to evaluate the impact of changes made to other aspects of radiotherapy pathway in other centers.

CONCLUSION

In this review we demonstrated that high-quality RWD has the potential to provide robust evidence in the field of RIHD. Although RCTs are generally not practical for the evaluation of cardiac toxicity, emerging evidence and new methodologies using RWD are providing an alternative to the classical RCTs. The RAPID-RT study will use RWD to assess the clinical impact of introducing a new cardiac avoidance region dose constraint with the aim of reducing the risk of RIHD and improving survival.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

FUNDING

This work did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

ACKNOWLEDGMENTS

This work was supported by the Cancer Research UK RadNet Manchester [C1994/A28701]. CF-F and MvH are supported by the National Institutes of Health Research of Manchester Biomedical Research Centre.

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frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 22 July 2022
DOI 10.3389/fonc.2022.931023

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OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Radiation Oncology, a section of the journal Frontiers in Oncology

RECEIVED 28 April 2022
ACCEPTED 28 June 2022
PUBLISHED 22 July 2022

CITATION
Wiedemann J, Coppes RP and van Luijk P (2022) Radiation-induced cardiac side-effects: The lung as target for interacting damage and intervention.
Front. Oncol. 12:931023.
doi: 10.3389/fonc.2022.931023

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Radiation-induced cardiac side-effects: The lung as target for interacting damage and intervention

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Radiotherapy is part of the treatment for many thoracic cancers. During this treatment heart and lung tissue can often receive considerable doses of radiation. Doses to the heart can potentially lead to cardiac effects such as pericarditis and myocardial fibrosis. Common side effects after lung irradiation are pneumonitis and pulmonary fibrosis. It has also been shown that lung irradiation has effects on cardiac function. In a rat model lung irradiation caused remodeling of the pulmonary vasculature increasing resistance of the pulmonary vascular bed, leading to enhanced pulmonary artery pressure, right ventricle hypertrophy and reduced right ventricle performance. Even more pronounced effects are observed when both, lung and heart are irradiated.

The effects observed after lung irradiation show striking similarities with symptoms of pulmonary arterial hypertension. In particular, the vascular remodeling in lung tissue seems to have similar underlying features. Here, we discuss the similarities and differences of vascular remodeling observed after thoracic irradiation compared to those in pulmonary arterial hypertension patients and research models. We will also assess how this knowledge of similarities could potentially be translated into interventions which would be beneficial for patients treated for thoracic tumors, where dose to lung tissue is often unavoidable.

KEYWORDS

lung, heart, cardiotoxicity, radiation, pulmonary hypertension, vascular remodeling

Introduction

According to the WHO (world health organization) cancer is one of the leading causes of death worldwide with around 10 million cases in the year 2020. Lung and breast cancer are the two most diagnosed cancer types (1). However, improvement of therapy in the last decades has enhanced survival significantly. Around 50% of all cancer patients

receive radiotherapy during their treatment (2), with lung and breast cancer amongst the leading indications (3). When treating thoracic tumors with radiotherapy, heart and/or lung tissue often receive substantial radiation doses.

As survival rates improve, irradiation effects of normal tissue become an increasing concern. Radiation-induced heart diseases (RIHD) are one of the major side effects after thoracic radiotherapy (4). Several recent studies have shown that the risk of cancer patients dying from cardiovascular events is higher than in the general population (5–7). Typical RIHDs that are seen are valvular disease, pericardial disease, conduction abnormalities, cardiomyopathy, and accelerated coronary artery disease (4). Several recent review articles have pointed to radiation-induced endothelial damage in the heart tissue as sign of cardiac injury, followed by the activation of several inflammatory pathways, the release of specific cytokines and immune cell activation which, in combination, finally lead to fibrosis (4, 8, 9). However, previous studies have mostly only investigated direct exposure of the heart or its substructures as a possible cause of RIHD. In this review, we aim to summarize evidence that irradiation of lung tissue also has an influence on the heart, especially on RV function, and that the interplay of effects on heart and lung contribute to radiation-induced cardiac toxicity. Furthermore, we hypothesize that the irradiation effects on lung tissue as well as the resulting cardiac effects show remarkable similarities to features of pulmonary arterial hypertension (PAH). The role of the lung in the development of cardiac side-effects suggests that this may offer novel targets for interventions to prevent such side-effects. Therefore, we aim to show parallels of the effects of lung irradiation with features observed in pulmonary arterial hypertension, and discuss possible subsequent treatment options.

Radiation effects on lung tissue contribute to cardiotoxicity

It is well known and reported that lung irradiation induces RILI (radiation induced lung injury) which is often divided in early acute toxicity manifesting as radiation pneumonitis and chronic toxicity resulting in pulmonary fibrosis (10–12). As primary mechanisms of damage, direct DNA damage and the generation of reactive oxygen species (ROS) are described. Radiation- induced apoptotic cell death of epithelial and endothelial cells, together with the effects of ROS, contribute to an inflammatory state leading to radiation pneumonitis (11). Cytokines and growth factors released during the inflammatory state enhance collagen production in fibroblasts, and finally lead to late phase pulmonary fibrosis (12).

However, in addition to these often-described effects of lung irradiation, vascular damage and vascular remodeling also occurs in the time frame of several weeks after irradiation. The loss of vascular endothelial cells and the consequent loss of barrier function is accompanied by the thickening of vessel walls, occlusion of small vessels and perivascular edema (13, 14). Vascular remodeling was observed after whole thorax irradiation, in several studies performed in small animals (15, 16). In addition, those studies report enhanced vascular resistance in the lung (15). Such increased resistance in the pulmonary vasculature leads to increased pulmonary artery pressure, which in turn can induce or worsen right ventricle remodeling (17) followed by a reduction of right ventricular performance. Table 1 summarizes the experiments performed in animal models providing evidence for this. Gosh et al. (16) showed that beside an increased vascular resistance and reduced vessel density, right ventricular hypertrophy could also be observed after whole thorax irradiation of rats with 10 Gy.

TABLE 1 Summery of preclinical animal studies showing evidence that vascular remodeling after irradiation of the lung can lead to cardiotoxicity.

Reference	Animal model	Irradiation modalities and dose	Irradiation field	Time points
Molthen et al., 2012 (15)	Female WAG/Rij/Cmcr rats	Photons, 10 Gy	Whole thorax	2 month after irradiation
Gosh et al., 2019 (16)	Female WAG/Rij/MCW rats	Photons, 10Gy	Whole thorax	Up to 12 month after irradiation
McChesney-Gilette et al., 1991 (18)	Adult beagle dogs, 1 year old	Photons, 12 Gy	Group I: entire heart and lung Group II: lung only, heart shielded Group III: entire heart+ overlying lung	Up to 24 weeks
Ghobadi et al., 2012 (19)	Adult male albino Wistar rats	Protons, 20Gy	33% lateral lung 50% lateral lung Lung excluding heart Heart+lung	8 weeks
Ghobadi et al., 2012 (20)	Adult male albino Wistar rats	Protons, 20Gy	Heart+ 25% lung Heart+ 50% lung 50% lung	8 weeks

Although the heart was included in the irradiation field, the authors speculate that lung irradiation may influence right-ventricle function. Testing this hypothesis requires precise irradiation of defined lung volumes, excluding the heart, which is challenging in small animals like rats and mice. Dogs were used in a study in the 1990s to achieve this. This study proposed that pulmonary hypertension is secondary to lung irradiation and is most likely followed by right ventricle hypertrophy (18). A study using high-precision proton irradiation to enable precise irradiation of different lung volumes showed that the irradiation of 75% of the lungs with 17 Gy, while shielding the heart, leads to significant increases in pulmonary artery and right ventricle pressure in combination with right ventricle hypertrophy and vascular remodeling in the lung (19). In the same study, irradiation of the whole lung was shown to lead to pronounced pulmonary dysfunction and vascular remodeling in the absence of major parenchymal remodeling. This highlights that these pulmonary vascular and right ventricle effects can occur at lower doses than parenchymal remodeling, making it a potentially relevant side effect that may be occurring in patients after radiotherapy. Moreover, in the same model, combined irradiation of lung and heart led to a stronger effect, which was explained by the interplay of the two organs in the cardiopulmonary system (20). Vascular remodeling in the lung tissue leads to enhanced resistance and contributes to right ventricular remodeling while the direct irradiation of the heart leads to a reduced diastolic function in the left ventricle. In summary, these preclinical data obtained in dog and rat models show that radiation effects in the lung can lead to cardiac side-effects, even if the heart is not in the irradiation field.

Pathology of vascular remodeling in PAH and as a result of irradiation

It has long been established in cardiology that vascular remodeling occurs during the development of pulmonary arterial hypertension (21). Pulmonary arterial hypertension is a progressive cardiovascular disease with a high mortality and a strong impact on the quality of life (22). The enhanced pulmonary resistance causes pressure overload of the right side of the heart leading to right-sided heart failure causing potential death. The disease is well-characterized and, although limited, treatment options are available. Interestingly, although the cause differs, the vascular remodeling process leading to right heart failure in PAH shows similarities to those observed after irradiation.

In healthy lungs, vessels consist of three layers: the intima, the media and the adventitia. The intima is the innermost layer consisting of one layer of endothelial cells (ECs). The medial vascular wall is mainly composed of smooth muscle cells (SMCs) which are quiescent in normal lung tissue. The adventitial layer is the outermost vessel layer. It mainly consists of fibroblasts, but also contains immune modulatory cells, resident progenitor cells, endothelial cells, and adrenergic nerves (23).Vascular remodeling observed in PAH is characterized by the thickening of the three layers of the vascular wall (21, 22, 24). Although deposition of extracellular components such as collagen can contribute to the thickening, the main component is hypertrophy and hyperplasia of cells in their corresponding layer. In addition, in peripheral arteries where precursor cells differentiate to SMCs, muscularization can occur. Interestingly, most of these pathological features of vascular remodeling typical for PAH can also be observed in a comparable manner after irradiation. In the following sections this is described in more detail for each layer of the vascular wall.

The intimal layer

One of the first stages in the development of PAH is apoptosis and dysfunction of endothelial cells. In PAH, endothelial apoptosis is mostly mutation-driven and apoptotic cell loss leads to the disruption of vessel lining and leakage of micro vessels (25). A change in the endothelial cells lining small and larger vessels is also shown in lung tissue after irradiation. A staining with the endothelial cell specific marker HIS52 shows endothelial cell detachment and loss after irradiation leading to a disruption of the endothelial layer (19). In general, apoptosis of endothelial cells is described for irradiation doses above 5 Gy in *in vivo* and *in vitro* experiments (13, 26). For PAH it is described that the remaining endothelial cells are apoptosis resistant and proliferate to regenerate the damage caused by the loss of apoptotic endothelial cells (21, 22, 25). Overshooting proliferation can subsequently lead to massive thickening of the intima and so-called neointima formation. Both are indeed also observed in irradiated lung tissue, contributing to the overall thickening of the vessel walls (19, 27) making it likely that some endothelial cells are resistant to radiation induced apoptosis, and triggering following processes in vascular remodeling (21, 25). Neointima formation and neointimal lesions are features of complex or advanced lesions, while more progressed lesions are called plexiform lesions (28). Neointimal lesions as well as plexiform lesions are characteristic for severe forms of PAH, are mainly observed in a late state of the disease and are often considered as a hall mark of PAH (22, 29). The above-mentioned rat model system of irradiation shows lesions comparable to those advanced lesions including neointima formation (19). The described changes and apoptosis in a subset of endothelial cells in PAH may have a different cause than after irradiation, but this comparison shows that the effects on the vascular wall are quite similar. Therefore, apoptosis and changes in ECs can be a driver of vascular wall thickening and remodeling.

The media

In PAH the transition of pulmonary arterial smooth muscle cells (PASMCs) to proliferating cells leads to remodeling and thickening of the medial vascular wall, and muscularization in smaller vessels. This often happens in parallel with the above described neointima-formation. Muscularization of small vessels and thickening of the medial layer is also described after lung irradiation in a rat model (19) shown in histological staining as well as in a significant enhanced thickness of the vessel wall together with vessel occlusion. In a rat model of whole thorax irradiation muscularization of a few vessels was observed already at a dose of 10 Gy (15). Proliferation of PASMCs can be caused by mediators released from endothelial cells (30). This is in line with the finding, that *in vitro* irradiation of SMC can lead to inhibition of proliferation and migration, which is used to counteract vascular stenosis (31). This result shows that muscularization, and therefore SMCs proliferation after irradiation, is most likely triggered by factors released from other cells. Senescence of PASMCs and related cytokine release can also play a role in the induction of proliferation (22) and will be discussed later. In addition, hypertrophy of the PASMCs and the enhanced deposition of extracellular matrix contribute significantly to the thickening of the media (29). As such, since thickening of the medial layer and muscularization of small vessels can be observed after irradiation, this is most likely the result of fibroblast activation due to the release of mediators by other cells.

The adventitia

In an early phase of the restructuring process of the vessel wall in PAH the activation of the fibroblasts in the adventitia and the induction of proliferation and reprogramming processes but also the onset of chemokine and cytokine release occurs. Although not playing a primary role in vessel dysfunction, mild adventitial thickening is known to occur in PAH. Even though not described explicitly in models of lung irradiation, it may be part of the overall observed thickening of the vascular wall in these models (16, 19). However, after coronary brachytherapy, used to counteract neointima formation and restenosis after cardiovascular interventions, a thickening of the adventitial layer has been observed as late adverse effect, even if intima hyperplasia could be inhibited successfully (32, 33). Furthermore, inflammation and activation of the peroxynitrit-PARP-pathway could be observed in the outer vessel wall after gamma irradiation of the carotid arteries (32). The function of the adventitial layer as a hub for inflammation will be discussed later. Adventitial thickening is not amongst the most important mechanisms in PAH. However, this layer of the vascular wall, including several different cell types, plays a crucial role in inflammatory processes and similar effects could be found after irradiation.

Taken together, the main pathological features of PAH - namely the loss of endothelial cells, the thickening of the intimal layer and neointima formation as well as medial thickening and muscularization - are also observed after irradiation. This supports the hypothesis that radiation may induce PAH and might be an overlooked side effect of thoracic radiotherapy. Recognizing PAH as a potential side-effect may open new opportunities to prevent or treat side-effects of thoracic radiotherapy by intervening in mechanisms playing a role in PAH.

Molecular mechanisms of vascular remodeling

Although the basic origin is different, there are many parallels in the molecular mechanisms involved in the initiation and progression of vascular remodeling in PAH and after lung irradiation. These include, for example, apoptosis, inflammation, and senescence. Although the chronological order is not completely clear, DNA damage and apoptosis is an initial step (34) (Figure 1). in PAH as well as after irradiation. In some of the PAH patients a genetic predisposition is a prerequisite for this (35). DNA damage and apoptosis is followed by the induction of senescence, inflammation and EndMT which are processes occurring most likely in parallel. The radiolysis of water is the main source of ROS after irradiation, occurring during the irradiation process and contributing to an environment favoring other processes such as inflammation (36, 37). In PAH, ROS play a role later in the process, increasing vasoconstriction but also contributing to vascular remodeling. In the following sections the mechanisms will be discussed separately to show parallels (Figure 2) playing a role in PAH induction and in vascular remodeling after irradiation.

DNA damage and apoptosis

Even if the exact timing is unclear, a dysfunctional EC phenotype occurs in parallel with the manifestation of PAH. Indeed, various data support that EC injury and apoptosis are involved in the early steps of PAH development. In an animal model, EC apoptosis has been shown to be a direct trigger for the induction of PAH (34). Apoptosis can have a number of different triggers. Around 10% of PAH patients have a genetic predisposition to develop the disease due to EC apoptosis-prone genotype. They carry heterozygous mutations in the bone morphogenetic protein receptor type 2 (BMPR2) (35), which normally mediates pro-survival signaling in endothelial cells. BMPR2-knock-out experiments in mice (38) showed that

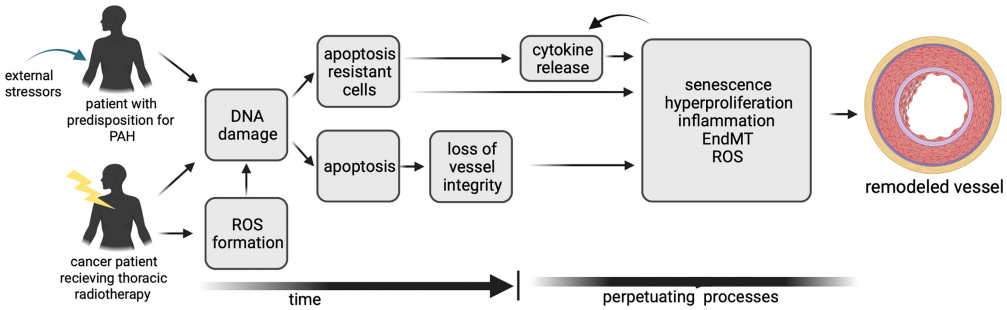

FIGURE 1
A timeline of the mechanisms contributing to vascular remodeling in PAH and after irradiation, illustrating differences and similarities.

the BMPR2 mutation might contribute to the initial step of EC apoptosis in PAH patients. However, unrelated to BMPR2 mutations, endothelial cells of PAH patients are also found to be intrinsically more prone to DNA damage (39). DNA damage can lead directly to apoptosis or initiate the activation of repair pathways. Cells of pulmonary arteries isolated from PAH patients show enhanced expression of the damage marker 53BP1 and γH2AX together with PARP-1 activation as part of the DNA damage response. PARP-1 activation can increase survival and proliferation, playing a role in the progression of PAH. In addition, DNA damage also downregulates BMPR2, decreasing the DNA damage response and further compromising genomic integrity of the cells.

Endothelial cell death has also been described after irradiation. *In vitro* experiments show significant levels of apoptotic cells in primary cultures of ECs derived from bovine adrenal micro vessels after irradiation with a dose of 10 Gy (26). Persistent radiation-induced DNA damage leads to p53 accumulation and caspase activation. EC apoptosis can also be modulated by the sphingomyelin/ceramide pathway. The phospholipid sphingomyelin which is present in the cell membrane is hydrolyzed upon TNF activation after irradiation leading to ceramide-mediated apoptosis of endothelial cells. Enhanced sphingomyelin levels have been shown in patients undergoing high-dose radiotherapy (40). Even if mediated by different pathways, DNA damage leads to apoptosis in PAH as

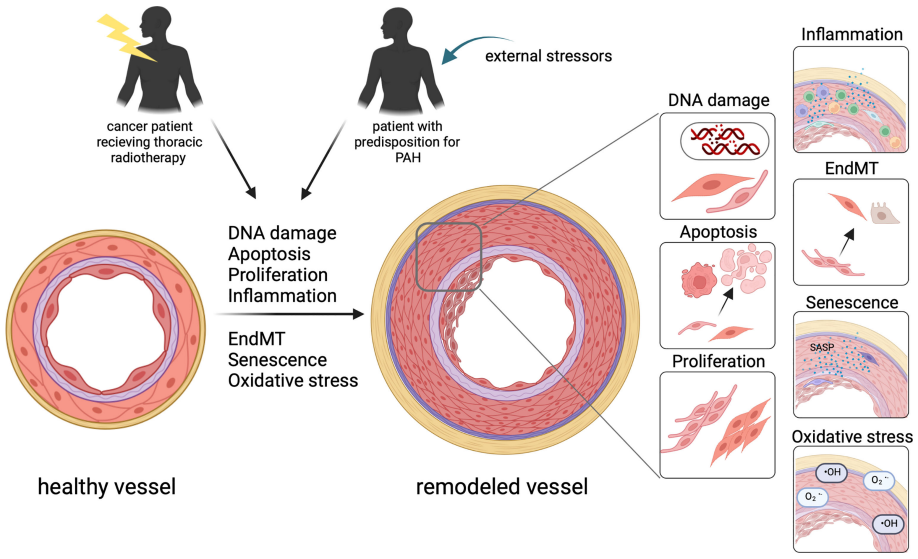

FIGURE 2
In patients with a predisposition for PAH as well as well as in patients after thoracic radiotherapy, similar mechanisms contribute to vascular remodeling turning a healthy vessel to a vessel with significant thickening of all layers of the vessel wall. This includes DNA damage, apoptosis, proliferation, inflammation, EndMT, senescence and oxidative stress contributing to the remodeling of the vessel.

well as after irradiation in endothelial cells and can serve as a critical early step in the initiation of the disease. Regardless of the actual origin, the loss of vessel integrity seems to be a starting point for a similar progression of vascular remodeling in PAH and after irradiation.

Senescence

Senescence is a process in which cells lose their capability of proliferation and cell growth and are arrested in G0/G1 phase (41). It is characterized by upregulation of senescence-associated genes and significant increase of specific P-galactose activity (42). Typically, senescent cells represent a senescence-associated secretory phenotype (SASP) which can contribute to the initiation and progression of several diseases in which senescence is involved (41). It has been shown that senescence of endothelial cells plays a role in the development and end stage of PAH (42). They show dysfunctional signaling and therefore reduced integrity, contributing to PAH development (38) and progression to an irreversible state (43). Furthermore, the study of van der Feen et al. (43) showed that treatment with the senolytic ABT263 enables the reversal of the hemodynamic and structural changes.

Senescence is also a very well-known phenomenon after irradiation, occurring in different cell types and known to contribute to normal tissue effects. After radiotherapy, healthy cells undergoing senescence contribute to the risk of early and late complications and morbidity as they can contribute to tissue fibrosis and organ dysfunction (44, 45). After lung irradiation senescent fibroblasts contribute with their activated SASP to radiation induced fibrosis (45). It has also been demonstrated *in vitro* and *in vivo* that lung endothelial cells change to a senescent phenotype after exposure to ionizing irradiation (46–49). *In vitro*, enhanced beta-galactosidase activity, typical for senescent cells, as well as an upregulation of senescence-related pathways was described in human pulmonary artery endothelial cells (HPAECs) and in human lung microvasculature endothelial cells (HLMVECs) after exposure to X-ray irradiation (46, 49). Inflammation-related genes, which are also involved in senescence are amongst the most-upregulated genes in these cells after irradiation (46) showing the activation of an SASP and also the relation and interaction of the two mechanisms.

Inflammation

Another hallmark in initiation, development and progression of PAH is inflammation. Endothelial cells are the source of key mediators for vascular remodeling including growth factors such as FGF-2, angiotensin II (AngII), vasoactive peptides and molecules like nitric oxide (NO), prostaglandin I2 (PGI2), endothelin-1 (ET-1) and pro-inflammatory cytokines like IL-6 and chemokines. Also, secretion of pro-inflammatory adhesion molecules e.g. ICAM1, VCAM and E-selectin from endothelial cells is enhanced. After irradiation-induced cellular damage, endothelial cells acquire a pro-inflammatory phenotype which leads to the release of similar factors as described for ECs in PAH (38). In addition to remodeling and thickening of the intimal layer, overproduction of these mediators also affects fibroblast-like cells and PASMCs in the vascular wall as well as endothelial cells in an autocrine way. In particular, endothelin-1 and IL-6 induce the differentiation from fibroblasts to myofibroblast, which are highly proliferative and proinflammatory. In addition, they produce collagen and other extracellular matrix proteins contributing to pulmonary vascular remodeling. The fibroblasts in the adventitia are suggested to sense vascular damage, leading to the activation of dendritic and progenitor cells resident in the adventitia to release key regulators of vascular remodeling (22, 50). Factors such as GM-CSF, CCL2 and CXCL12 promote the recruitment of leukocytes, which in turn release factors inducing endothelial cell apoptosis or the switch to the pro-inflammatory phenotype of ECs (22, 23, 38, 50). In a later stage of PAH, immune cells, mainly T cells, monocytes and macrophages infiltrate the vascular wall. They regulate the fate of other vascular cell types *via* direct or indirect signaling and are therefore the main driver and regulator for the remodeling of all parts of the vessel walls by triggering activation, migration, differentiation, proliferation and survival of ECs, SMCs and fibroblasts (21).

Interestingly, in a mouse model, lung specific overexpression of IL-6 was sufficient to induce remodeling of the vasculature, mainly muscularization of arteries and neointima formation, leading to enhanced RV systolic pressure and RV hypertrophy (51). Also, in PAH Patients, enhanced serum levels of IL-6 have been observed, underlining the role of IL-6 as an important mediator in PAH (22). Both *in vitro* and *in vivo* experiments demonstrated that irradiation leads to increased IL-6 release from endothelial cells and fibroblasts (52–54).

The described release of inflammation related mediators from different cell types in PAH as well as after irradiation, show that in both cases a pro-inflammatory milieu is established which contributes to the progression of changes in the vascular walls and vascular remodeling. With the radiation-induced release of IL-6 from endothelial cells and fibroblasts, a key mediator and initiator for PAH associated processes is present.

Endothelial to mesenchymal transition

Another important process in the development of PAH is the transition of activated endothelial cells into cells expressing markers of SMCs or mesenchymal cells (EndMT), explaining the presence of alpha-smooth-muscle-actin positive cells in the media (55, 56). During the process of the transition

endothelial cells lose the expression of the endothelial cell marker CD31 and cadherin, gap junctions are lost, the cells dissociate from the basement membrane and start to migrate into the medial layer. During this process they start to express markers such as alpha-smooth muscle actin or vimentin (56). The transformed cells fail to form an intact barrier and are primed for proliferation and migration, potentially contributing to the formation of neointimal lesions (57). In addition, they can acquire a pro-inflammatory phenotype, after which their release of cytokines contributes to the inflammatory milieu in PAH. In PAH, EndMT is induced through the Smad2/3, Erk1/2 and p38MAPK pathways but can also occur in response to inflammatory cytokines like IL-1beta and TNF-alpha.

Interestingly, EndMT is also induced after radiotherapy (58). In normal tissue, radiation-induced EndMT is mainly recognized as a driver for radiation induced pulmonary fibrosis (RIPF). However, it also contributes directly to vascular remodeling in the early phase of RIPF development and is a process occurring prior to mesenchymal transition of alveolar epithelial cells. *In vitro* experiments using human pulmonary microvascular endothelial cells (HPMVECs) showed an upregulation of mesenchymal markers accompanied by a downregulation of EC markers in RNA sequencing data after exposure to ionizing radiation (46). In line with this, EndMT is dependent on TGF-betaR1 and Smad signaling in an *in vitro* model after irradiation (58). This is similar to the activation processes in PAH. In addition, radiation induced EndMT of ECs promotes the transition from fibroblasts to myofibroblasts which also contribute to vascular remodeling (59). In conclusion, EndMT occurs in endothelial cells in PAH, but it is also a well-known irradiation-induced process, not only contributing to radiation induced fibrosis but also to vascular remodeling. This again shows the parallels in both mechanisms.

Reactive oxygen species and oxidative stress

Reactive oxygen species (ROS) summarizes a group of reactive molecules, including free radicals, superoxide (O_2−), hydroxyl (•OH−) and hypochlorite (OCl−), but also non-radical species such as hydrogen peroxide (H_2O_2) and other peroxides (ROOH) which can oxidize other molecules (60, 61). An imbalance due to enhanced ROS production and reduced production of antioxidants leads to oxidative stress (62). It has been shown in patients as well as in animal models that oxidative stress and increased ROS levels play an important role in the development of PAH (61–64). Oxidative stress contributes to PAH in different ways: It interferes with production of vasodilators like NO and PGI2 from vessels by damaging the endothelial nitric oxide synthase (eNOS) and prostacyclin synthase (PGIS) leading to enhanced vasoconstriction (64). It also promotes vasoconstriction by enhancing endothelin-1 release from endothelial cells and consequent activation of the endothelin-1 pathway (65). In addition, oxidative stress contributes to vessel thickening as it activates the production of transforming growth factor-β1 (TGF-β1), vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2) and platelet-derived growth factor (PDGF) (62). Macrophages, ECs, PASMCs and fibroblasts are sources of ROS and oxidative stress in PAH as nicotinamide adenine dinucleotide phosphate (NADPH) oxidases are found in those cells (66, 67). The NADPH oxidases 1-5 play an important role in lung vasculature by increasing ROS generation which contributes to vascular dysfunction and in turn can lead to further ROS production (62). Other sources of ROS in PAH are the xanthine oxidase (XO) and nitric oxide synthases. During disease state, mitochondria electron transport complexes can also be disrupted and become a source of ROS. However, NADPH oxidases are accepted as the major source of ROS in lung vasculature and are thought to regulate the other sources (64).

ROS production and oxidative stress contribute significantly to normal tissue damage after exposure to ionizing radiation. Radiolysis of water leads to the production of free radicals with very short half-lives immediately after irradiation in the extracellular environment (36, 37). In addition, the redox system contributes to radical production in the time frame of hours after irradiation and can last for years (36). Like in PAH, upregulation of enzymes such as NADPH oxidases play a major role in ROS production. These enzymes have effects on mitochondrial function leading to further ROS production (36) in leukocytes and macrophages but also e.g., in fibroblasts (68). Oxidative stress and ROS in turn, leads to DNA damage which can be followed by apoptosis, but can also contribute to senescence. These processes lead to the secretion of inflammatory cytokines and can therefore contribute to normal tissue damage. As discussed above, these mechanisms are also known to contribute to vascular remodeling in lung tissue which can lead to PAH induction and progression.

Hypoxia

The relation between hypoxia and PAH is very well established (69). Hypoxia plays a major role in several animal models of PAH, either as chronic exposure to hypoxia alone or in combination with other components e.g., Sugen, a VEGFR-2 antagonist (63). In chronic hypoxic models, where mainly rats but also other animals are exposed to 10-12% oxygen for several days or weeks, pulmonary artery remodeling as well as remodeling of smaller vessels occurs and RV hypertrophy is observed (64). In combination with a single Sugen injection, more severe PAH is developed (63). The combination with hypoxia enhances the effects of overexpression of IL-6 in the lung and leads to a more severe PAH in a rat model (51). In

patients, hypoxia may not be a primary reason to develop PAH, but hypoxia contributes to the risk of developing PAH, especially in people with preexisting lung pathologies (70). However, as shown in the animal models, hypoxia can act as prerequisite for the induction of PAH or, in chronic hypoxia be the main inducer of PAH (63, 64).

It has been shown that ionizing radiation can induce hypoxia in the lungs (71). Vujaskovic et al. (71) investigated hypoxia in a rat model after hemithorax-irradiation with 28Gy and found moderate levels of hypoxia in the lungs 6 weeks after irradiation and more severe levels after 6 months. Fleckenstein et al. (72) showed reduced perfusion of the lung early after irradiation in the same model system accompanied by hypoxia, oxidative stress, and enhanced cytokine production. Radiation-induced hypoxia in lung tissue is mainly thought to be related to lung fibrosis; however, vascular remodeling is also shown as a result (72). Hypoxia occurring after irradiation in lung tissue can contribute to an environment which favors the development of PAH, similar to that described for hypoxic animal models of PAH. Furthermore, hypoxia can contribute to ROS formation and activation of other previously discussed mechanisms which contribute to PAH initiation and development.

Possible interventions

Even if described as limited, treatments for PAH are available in cardiology practice for non-oncological patients. Fourteen FDA approved drugs treating PAH are on the market (73). New drugs, targeting other pathways and mechanisms in PAH are under development and some of them have already been tested in clinical trials (73). As summarized in the previous sections, there are many similarities in the mechanisms leading to pulmonary arterial hypertension and those involved in radiation-induced vascular damage. In line with this, we suggest that treatment options which are available for PAH may potentially also be able to counteract initiation and progression of PAH-like features induced by irradiation. This suggests that the effectiveness of these drugs could be investigated preclinically and subsequently clinically to potentially yield new interventions to specifically prevent or treat pulmonary vascular damage. Therefore, in the following sub-sections current drugs and current developments in the cardiology field that may be of interest for the reduction or prevention of cardiopulmonary damage due to irradiation will be discussed (Figure 3).

Approved drugs for PAH treatment

Approved drugs for PAH can be classified into 4 classes: prostacyclin analogs, endothelin-receptor antagonists (ERAs), phosphodiesterase type 5 (PDE-5) inhibitors and soluble guanylate cyclase (sGC) stimulators. The latter target 3 pathways: excess endothelin (ET) activity, abnormal nitric oxide (NO) activity, and prostacyclin (PGI2) deficiency (73). A combination of an endothelin receptor antagonist and a phosphodiesterase 5 inhibitor is currently widely accepted as standard of care therapy (74). These include, for example, the PDE-5 inhibitors Sildenafil or Tadalafil in combination with Bosentan, Macitentan or Ambrisentan. The available treatments mainly focus on vasodilation and not primarily on the reduction of vascular remodeling (75). However, it has also been shown that vascular remodeling is reduced after treatment with these drugs. The similarities in the mechanisms and events leading to vascular remodeling suggests that the treatment with these medications or a combination of those could potentially be a tool to counteract or prevent the development of PAH in radiotherapy patients and should be further explored.

New treatment options for PAH

Besides the described routinely used medications, other medical approaches targeting different mechanisms are currently under development for PAH patients. These were recently reviewed in e.g., Sommer er al (76). and Condon et al. (73). For example, modulating the BMP signaling, targeting sexual hormone related pathways or other hormonal pathways are considered as promising tools for PAH treatment. However, these mechanisms may not play a role after irradiation. Here we aim to summarize treatment options which are a potential option for radiotherapy patients and therefore target mechanisms in which parallels were identified in the previous sections.

Altered growth factor signaling is involved in the characteristic hyperproliferation and apoptosis-resistance of endothelial and smooth muscle cells in vascular remodeling of pulmonary arteries in PAH as well as in radiation-induced vascular remodeling. As this signaling involves tyrosine kinase receptors, the use of tyrosine kinase receptor inhibitors is currently tested. An oral tyrosine kinase receptor inhibitor, matinib mesylate, showed promising results in terms of hemodynamic improvement in a stage 3 clinical trial, but had significant side effects (77). Therefore, another clinical trial which aims to find a tolerable dose and treatment regime is currently recruiting (78). In addition, a more specific tyrosine kinase inhibitor binding to PDGF receptors is currently being tested (73).

Another promising approach is the autologous treatment with patient-derived endothelial progenitor cells which are transfected with the endothelial nitric oxide synthase gene. Nitric oxide is a potent vasodilator which is normally synthesized by the (eNOS) and released from endothelial cells. In PAH, as well as after irradiation, the loss of endothelial cells is an initial step of vascular remodeling. In addition, in PAH a

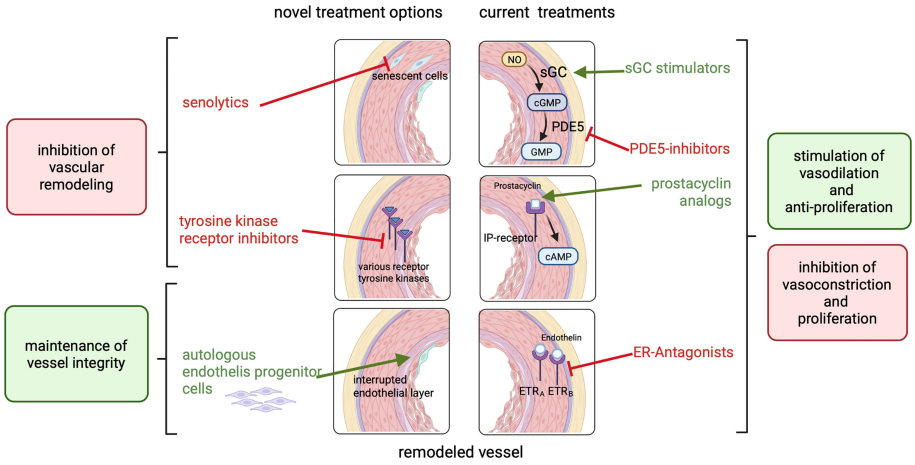

FIGURE 3
Currently approved treatments for PAH (right side) include ER-antagonists, prostacyclin analogs, PDE5-inhibitors and sGC stimulators which inhibit vasoconstriction and proliferation or act vasodilative and anti-proliferative to reduce blood pressure and inhibit vascular remodeling. Potential novel treatment options (left side) like senolytics or tyrosine kinase receptor inhibitors inhibit vascular remodeling. The treatment with patient-derived endothelial progenitor cells can maintain vessel integrity.

reduction of eNOS expression has been shown in the remaining endothelial cells, leading to low levels of NO. Several studies have shown that administration of EPCs restores endothelial function due to vessel repair and promotes angiogenesis by releasing proangiogenic factors (79, 80), while eNOS-transfected cells improved hemodynamic and RV function (81, 82). For PAH patients, this approach is currently tested in a clinical trial. Because the loss of endothelial function represents one of the main steps in vascular remodeling, this treatment approach could be considered for radiotherapy patients. Further investigation would be necessary to check whether eNOS expression in endothelial cells is also reduced after irradiation and treatment with eNOS transfected cells would lead to an additional improvement.

As previously discussed, senescence is another relevant mechanism in both PAH development and after irradiation exposure. Van der Feen (43) showed that a switch from a proliferative to a senescence phenotype in vascular endothelial cells is related to the loss of reversibility in a PAH model. The use of senolytics such as ABT263 proved that removal of senescent cells can facilitate the reversal of haemodynamic changes, as well as structural changes which are present in severe PAH. Although not yet tested clinically, senolytics are currently considered as a potential treatment to counteract adverse effect of normal tissue irradiation (83, 84). Treatment with senolytics after thoracic irradiation may be a promising tool to counteract vascular remodeling and consequent PAH. In addition, the data from van der Feen (43) indicate that reversal of already present PAH is possible if senescence cells are eliminated. Beside the removal of senescent cells, the avoidance of senescence could also be a strategy to reduce side effects after irradiation. Experiments performed at very high dose rates (FLASH irradiation) have shown reduced normal tissue toxicity compared to conventional irradiation (85, 86). Interestingly, the reduced damage to normal tissue is at least in part due to reduced senescence induction, but this requires further investigation. Therefore, modulating the dose rate can be a strategy to reduce radiation induced side effects, accompanied by a reduction of senescence which will also reduce vascular remodeling. In addition, it has been shown that FLASH irradiation also reduces DNA damage in normal tissue which can also contribute to reduce normal tissue effects.

Discussion

We summarized evidence that loss of cardiopulmonary function due to vascular remodeling similar to that in pulmonary arterial hypertension may play an important role as a side effect of thoracic radiotherapy and we discussed possible novel interventions.

To this end we first showed that there is evidence in preclinical experiments for the occurrence of vascular remodeling in the lungs and consequent right ventricular effects similar to those in pulmonary arterial hypertension after exposure to ionizing irradiation. Next, we established various parallels in the mechanisms playing a role in the induction and development of PAH, and those induced after irradiation. Furthermore, we summarized classical as well as novel treatment options which are used in cardiology praxis to treat PAH and could potentially be used for radiotherapy patients.

This raises the question of what is needed in order to use the knowledge on treatment options of PAH and implement it in the standard pre and/or post-treatment care of radiotherapy patients.

First, in radiotherapy practice, vascular remodeling leading to PAH has not yet been recognized as a possible side effect of radiotherapy. More insights into the effects of irradiation on lung vasculature and the right heart are needed to prove that the preclinically observed effects also occur in patients. Several patient studies show that overall survival is related to lung and/or heart dose (87–89)while some cases of death could not be related to known cardiopulmonary side effects after irradiation, such as pneumonitis or fibrosis (87). This suggests that unrecognized side effects e.g. vascular remodelling leading to PAH-like symptoms, may be involved. However, up to now, the follow up on radiotherapy patients is restricted to classical endpoints of RILT and RIHT, such as fibrosis or lung inflammation. Detection of early PAH-related symptoms in patients requires different diagnostics in the routine follow-ups of radiotherapy patients. For example, echocardiography to check for RV performance and a more specific blood diagnostic checking for specific markers is needed to detect PAH-related changes. The CLARIFY study is the first study investigating more specific PAH related endpoints in radiotherapy patients (90). In this large prospective cohort study lung and esophageal cancer patients receiving thoracic radiotherapy are included. Echocardiography, cardiac MRI and the detection of blood biomarkers is performed to gain more insights into changes potentially related to PAH.

Besides establishing that PAH also occurs in radiotherapy patients, proof of concept is needed to show that interventions are also effective if PAH is induced by radiation. For this, further preclinical studies are needed to test medications approved for PAH in an animal model developing radiation-induced vascular remodeling. There are only very few studies testing e.g., a PDE-5 inhibitor like Tadalafil or Sildenafil to counteract irradiation-induced effects on the lung or the heart (91, 92), and these studies do not address effects on vascular remodeling but instead, for example, check for changes in miRNA levels. Information on the effect of those interventions on vascular remodeling and endpoints such as RV performance is required to assess the potential of these known interventions for PAH for testing in radiotherapy patients.

However, besides the parallels, there are also differences between patients developing radiation-induced pulmonary vascular remodeling and PAH patients. The most obvious difference is that radiotherapy patients receive their treatment because they have cancer. Therefore, when considering any interventions or medication to treat radiation induced PAH, it is important to verify that this does not lead to tumor protection. However, some medications used for the treatment of PAH, such as different endothelin inhibitors and antibodies (93)or PDE-5 inhibitors like Sildenafil (94–96) have been suggested as potential treatment option for cancer. Overexpression of PDE-5 has been reported for lung and breast cancer. Sildenafil is pre-clinical but is thought to not only enhance the sensitivity of different tumor cells to chemotherapeutic agents and reversing multidrug resistance, but also show anti-cancer effects itself (97). The endothelin-axis and especially ET-1 and its receptor are known to be hyperactivated and overexpressed in many malignancies and play a pro-hyperproliferative and pro-survival role (98). Clinical trials to prove this hypothesis are still lacking, but everal trials aiming to answer this question are ongoing (97–99). For other treatment options, effects on the tumor itself need to be considered as well.

Interestingly, lung cancer patients are at increased risk of cardiopulmonary comorbidities (100). In fact, there is evidence that pulmonary hypertension occurs with a higher incidence in those patients (101). For lung cancer, as well as for PAH, similar risk factors related to life-style, such as smoking and being overweight have been identified (102, 103) in addition to genetic predispositions. These are also factors which have to be considered in future approaches to prevent or treat irradiation-induced vascular remodeling.

Conclusion

There is preclinical evidence that radiation-induced vascular remodeling in the lungs can cause a reduction in right ventricular function. Recognizing similarities between vascular remodeling after irradiation and in PAH and the target cells and mechanisms involved, can open novel avenues for pharmacological prevention or treatment of this severe side-effect of radiotherapy for intra-thoracic tumors.

Author contributions

JW wrote the first draft of the manuscript and designed the figures. RPC and PvL provided critical feedback and proofread the manuscript. All authors contributed to the article and approved the submitted version.

Funding

JW and PvL received funding from the Dutch Cancer Society under grant number 12134 (AVERT) and 11349 (CLARIFY).

Acknowledgments

Figures were created with biorender.com.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 16 September 2022
DOI 10.3389/fonc.2022.940127

Check for updates

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Radiation Oncology, a section of the journal Frontiers in Oncology

RECEIVED 10 May 2022
ACCEPTED 29 August 2022
PUBLISHED 16 September 2022

CITATION
Irabor OC, Nelson N, Shah Y, Niazi MK, Poiset S, Storozynsky E, Singla DK, Hooper DC and Lu B (2022) Overcoming the cardiac toxicities of cancer therapy immune checkpoint inhibitors. *Front. Oncol.* 12:940127. doi: 10.3389/fonc.2022.940127

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Overcoming the cardiac toxicities of cancer therapy immune checkpoint inhibitors

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Immune checkpoint inhibitors (ICIs) have led recent advances in the field of cancer immunotherapy improving overall survival in multiple malignancies with abysmal prognoses prior to their introduction. The remarkable efficacy of ICIs is however limited by their potential for systemic and organ specific immune-related adverse events (irAEs), most of which present with mild to moderate symptoms that can resolve spontaneously, with discontinuation of therapy or glucocorticoid therapy. Cardiac irAEs however are potentially fatal. The understanding of autoimmune cardiotoxicity remains limited due to its rareness. In this paper, we provide an updated review of the literature on the pathologic mechanisms, diagnosis, and management of autoimmune cardiotoxicity resulting from ICIs and their combinations and provide perspective on potential strategies and ongoing research developments to prevent and mitigate their occurrence.

KEYWORDS

immune checkpoint inhibitor (ICI), Cardiotoxic adverse effect, immunotherapy, anti PD-1 antibodies, anti CTLA-4 antibodies, anti PD-L1 therapy, Cardiotoxicities

1 Introduction

In the past few decades, advances in cancer immunotherapy have revolutionized the management of metastatic and advanced-stage malignancies, improving survival in multiple cancers with abysmal prognoses prior to their introduction. On the frontline of these advances are the immune checkpoint inhibitors (ICIs), known to target immune checkpoints, which are critical immune system regulators that can dampen an immune response to a stimulus such as an infection. These inhibitory effects are essential to maintain

self-tolerance and prevent over activity of the immune cells. However, tumors exploit these regulatory pathways to escape T cell-mediated antitumor immunity. Tumor cells express ligands for immune checkpoint proteins such as the cytotoxic T lymphocyte-associated protein 4 (CTLA-4 also known as CD152), the programmed cell death 1 (PD-1 also known as CD278), and Lymphocyte Activation Gene-3 (LAG-3 also known as CD223) receptor molecules expressed on T lymphocytes. Tumor-expressed ligands activate these receptors, diminishing T-cell responses against the tumor. ICIs currently utilized in clinical practice are monoclonal antibodies that target these molecules: CTLA4, PD-1, PD-L1 (Programmed death ligand -1) and more recently LAG-3. These therapeutics block the receptor-ligand binding and release the inhibitory signaling, allowing T cells to continuously recognize and attack Tumor cells. The survival benefit of ICIs has been demonstrated in multiple randomized clinical trials, making them a mainstay therapy for various tumors. However, they are not without trade-offs. The remarkable efficacy of ICIs is limited by their potential autoimmune and inflammatory side effects known as immune-related adverse events (irAEs). IrAEs occur in about two-thirds of ICIs recipient requiring cessation of therapy in nearly 40 percent of patients (1). Autoimmune toxicities involve multiple organ systems such as the skin, gastrointestinal tract, liver, lungs, and endocrine system. Fortunately, most systemic, and organ-specific irAEs present with mild to moderate symptoms that can resolve spontaneously, with discontinuation of therapy or glucocorticoid therapy. In contrast to other organ-specific IrAEs, cardiotoxicities are rare, albeit with a high case fatality when they occur (1, 2). For example, the incidence of myocarditis in patient receiving ICI therapy ranges from 0.04% to 1.14%but with an associated mortality of 25% to 50% (3, 4). The potentially fatal outcome of cardiac irAEs warrant prompt intervention with supportive care and glucocorticoid therapy. Unfortunately, the rareness of this condition makes it difficult to obtain sufficient data and knowledge about these serious adverse events to form strategies for early detection, assessment, and management. As a result, the understanding of autoimmune cardiotoxicity remains limited, although rapidly evolving. In this paper, we provide an updated review of the literature on the pathologic mechanisms, diagnosis, and management of autoimmune cardiotoxicity as a result of ICIs and their combinations, and provide perspective on potential strategies and ongoing research developments to prevent and mitigate their occurrence.

2 Immune checkpoint inhibitors

There are at least nine US Food and Drug Administration-approved ICIs as of early 2022. These include an anti-CTLA4 monoclonal antibody (Ipilimumab); four PD-1 blocking monoclonal antibodies (Nivolumab, Pembrolizumab, Cemiplimab, and Dostarlimab); and three anti-PD-L1 antibodies (Atezolizumab, Durvalumab, and Avelumab) and one LAG-3 antibody (Relatlimab). Table 1 shows clinical indications of each ICI approved by the FDA. Tremelimumab, an anti CTLA-4 monoclonal antibody has an orphan drug designation and is currently under investigation as a combination regimen with other ICIs (clinicaltrial.gov). In addition, some newer anti-PD-1 ICIs, such as Sintilimab, Tislelizumab, Toripalimab, and Camrelizumab, which the National Medical Product Administration of China has approved, are currently undergoing Phase II/III testing. Some emerging anti-PD-L1 currently under investigation include Cosibelimab, KN035, CA-170, BMS-986189, etc. (5) .

All ICIs exert their antitumor activity by reversing the T cell tolerance towards tumor cells that is mediated by their checkpoint proteins. The mechanism of their toxicities,

TABLE 1 Current FDA approved Immune Checkpoint Inhibitors and their indications.

Drug	Target	FDA Indication	FDA approval Year
Ipilimumab	CTLA-4	Melanoma, colorectal cancer, renal cell carcinoma	2011
Nivolumab	PD-1	Melanoma, Head and neck squamous cell carcinoma, Non-small cell lung cancer, Small cell lung cancer, Hodgkin's lymphoma, Hepatocarcinoma, colorectal cancer	
Pembrolizumab	PD-1	Melanoma, non-small cell lung cancer, non-squamous cell lung cancer, renal cell carcinoma, classic Hodgkin's lymphoma, gastric or gastroesophageal junction adenocarcinoma, urothelial carcinoma, cervical cancer, large B-cell lymphoma, Merkel cell carcinoma	2014
Cemiplimab	PD-1	Cutaneous squamous cell carcinoma	2018
Dostarlimab	PD-1	Recurrent Endometrial cancer	2021
Avelumab	PD-L1	Merkel cell carcinoma, urothelial carcinoma, renal cell carcinoma	2015
Atezolizumab	PD-L1	Urothelial carcinoma, non-small cell lung cancer, breast cancer, non-squamous non-small cell lung cancer, small-cell lung cancer	2016
Durvalumab	PD-L1	Urothelial carcinoma, non-small cell lung cancer	2016
Relatlimab	LAG-3	Advance and metastatic melanoma	2022

including cardiac toxicity, relates to this process. Thus, understanding T cell activation and their inhibition is needed to understand ICIs toxicities.

2.1 Modulators of T lymphocyte activation and tolerance

T lymphocytes serve as one of the prime mediators of the adaptive immune response against tumors. T cell immune checkpoint receptors are a wide variety of molecules found on T cells that are known to modulate the signaling pathways involved in the activation of antigen-specific, including anti-tumor responses (6, 7) . Activating T cell receptors include the T cell receptor complex and costimulatory molecules such as CD28, OX40, GITR (Glucocorticoid-induced TNF receptor family-related protein), CD137, CD27, HVEM (herpesvirus entry mediator). Inhibitory T-cell receptors that mitigate against T cell activity include but are not limited to CTLA-4, PD-1, LAG-3 (lymphocyte activation gene-3), TIM-3 (T cell immunoglobulin and mucin domain-containing protein 3), BTLA (B- and T-cell lymphocyte attenuator), and VISTA (V-domain Ig Suppressor of T-cell Activation) and the TIGIT (T cell immunoglobulin and ITIM domain). The capacity to develop an immune response is largely a consequence of the balance of stimulatory versus inhibitory signaling which can result in autoimmunity, as seen in cardiac pathologies following ICI treatment. There are other lesser understood intracellular metabolic pathways such as the indoleamine 2, 3-dioxygenase (IDO), and arginase in tumors and myeloid cells that also play a critical role in activating immune cells (8). More also, some other immune checkpoints are now known to play a critical role in the modulation of other subsets of immune cells aside of T cells (e.g., CD40 for B cells and TIGIT for NK cells) (9, 10). However, the current clinically utilized ICIs exploit the membrane-bound immune checkpoint proteins (CTLA-4, PD-1, PD-L1, and the more recent LAG-3). Cardiotoxicities from these immunotherapeutic are, therefore, our focus in this review.

2.2 Mechanism of immune checkpoint inhibition

T lymphocyte activation involves the following steps. First, antigen-presenting cells (APCs) process antigens to load antigenic peptides onto their major histocompatibility complex (MHC) molecules for recognition by a T cell that displays a cognate T cell receptor (TCR) and a co-stimulatory CD28 receptor for B7-1 (CD80)/B7-2(CD86) expressed by the APC (7). This primarily occurs either in lymphoid tissues for priming or peripheral tissues for secondary responses. In lymphoid tissue, T cells are activated when their TCRs bind to their cognate MHC-peptide complex presented by APCs in conjunction with concurrent CD28 binding to B7-1/B7-2. This initial response to antigen causes induction of CTLA-4 within the T cells, which is contained within intracellular vesicles of naive T cells and is then transported to the cell surface and expressed as a membrane molecule. The membrane-bound CTLA-4 signals to dampen and maintain a controlled level of T cell activation. T cells stimulated in peripheral tissues mainly express PD-1 rather than CTLA-4. Unlike CTLA-4, PD1 expression is upregulated transcriptionally at the mRNA level in response to inflammatory signals (such as IFN-γ) that are produced by activated T cells (7).

CTLA-4 4 is a CD28 homolog with a stronger binding affinity for B7 that CD28. In the later phases of an immune response, membrane-bound CTLA-4 interacts with the B7 molecules on APCs, blocking their interaction with CD28 and thereby decreasing the T cell activation state which can render the cells anergic. Similarly, PD-1 binds to ligand PD-L1 and PD-L2 on the APCs to inhibit T-cell reactivity. Excessive induction of PD-1 on T cells in the setting of chronic inflammation and antigen exposure have been observed to cause T cell anergy. Figure 1 shows the CD28/CD80, CTL4/CD80 and PD1/PDL1 inhibitory ligand interaction. LAG-3 inhibits activation of T cells in a similar fashion to CTLA-4 and PD-1. It is co-expressed with PD-1 in activated T cells, natural killer (NK) cells and APCs with its main ligand is the MHC class II, to which it binds in place of CD4 (a receptor of TCR) to dampen T cell activation (11, 12) . These dampening effects are needed in normal physiologic conditions to prevent T cell over-activity and maintain self-tolerance during a T cell response to invading pathogens and other antigen sources. However, tumors exploit these regulatory pathways by expressing these inhibitory ligands thereby interfering with the ability of T lymphocytes to direct anti-tumor immunity. These inhibitory processes can be reversed by ICIs to promote cancer immunotherapy. Anti CTLA-4, PD-1/L1 and LAG-3 antibodies restore the activity of anti-tumor T cells through blocking CTLA-4/B7, PD1/L2-L2, and LAG-3/MHC class II interactions respectively. However, the precise understanding of the immunostimulatory mechanisms of various ICIs remain under investigation. For example, recent pre-clinical studies implicating CTLA-4 as an intrinsic positive regulator of regulatory T cell (Treg) as opposed to merely a negative regulator of T effector cells are noteworthy (13) and LAG3 blockade have also been shown to interfere with the suppressive activity of Treg cells (11).

2.3 Clinical benefit of immune checkpoint inhibition

Anti CTL4-A therapy: Ipilimumab prolonged overall survival (OS) in patients with stage III or IV melanoma in a clinical trial, leading to its approval in 2011 (14). A combination therapy of Ipilimumab and Nivolumab, which targets PD-1, was subsequently approved for melanoma following data from the Checkmate 067 trial, which demonstrated an OS benefit for the

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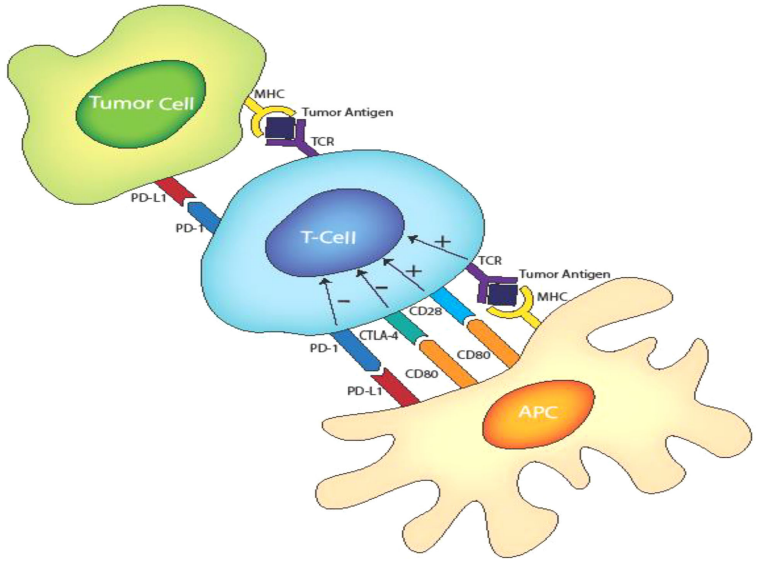

FIGURE 1
T cell activation and inhibitory receptors-ligand interactions involving TCR/MHC class II. CD28/D80. CTLA-4/CD80 and PD1/PD-L1.

combination therapy versus Ipilimumab monotherapy (15). It's indication further expanded to include renal cell carcinoma after the Checkmate 214 trial showed significant improvement in OS and progression-free survival (PFS) (16, 17). In the Checkmate 227 and Checkmate 9LA, Nivolumab plus ipilimumab as first-line treatment improved OS compared to chemotherapy in non-small cell lung cancer (NSCLC) (18, 19). The newer anti-CTL4A-4 Tremelimumab was granted an orphan drug designation after showing modest clinical efficacy for treating malignant mesothelioma in a phase II trial. Tremelimumab, however, failed to meet clinical endpoints in the DETERMINE trial (20, 21). Tremelimumab is currently tested for other tumor types and in combination therapy (22).

Anti PD-1 therapy: Nivolumab was approved by the FDA in 2014 based on the CheckMate-037 trial, which demonstrated an improvement in overall response rate with Nivolumab against standard-of-care chemotherapy in patients with advanced and progressing unresectable/metastatic melanoma (23). Its clinical use in melanoma has expanded since 2014 based on the Checkmate 067 and Checkmate 238, which demonstrated OS and PFS benefits combined with Ipilimumab (15, 24). Similar efficacy has been demonstrated for other disease sites. These include Checkmate 17/57 and CheckMate-032 trial (NSCLC) (25, 26) Checkmate-214 (Renal cell carcinoma), (17, 27) Checkmate-205 (Hodgkin Lymphoma), (28) Checkmate 275 (Urothelial carcinoma), (29) Checkmate-040 (hepatocellular carcinoma) (30) and Checkmate-141 (head and neck tumors). (31) Pembrolizumab combination superiority over prior standard of care in the KEYNOTE-407 and KEYNOTE-042 trial (NSCLC) (32, 33) KEYNOTE 181 (Esophageal cell carcinoma) (34), KEYNOTE-158 (metastatic small cell lung cancer) (35), KEYNOTE-426 (Renal cell carcinoma) (36), KEYNOTE-224 (Hepatocellular carcinoma), KEYNOTE-017 (Merkel cell carcinoma) (37), KEYNOTE-170 (B-cell lymphoma) (38), KEYNOTE-158 (Cervical cancer) (35) (39), KEYNOTE-059 (Gastric and gastroesophageal junction cancer) (39), KEYNOTE-158(MSI-h dMMR cancers) (40), KEYNOTE-048 (Head and neck cancers) (41), KEYNOTE-087 (Hodgkin lymphomas) (42), KEYNOTE-006 (Melanoma) (14), and KEYNOTE-045 (Urothelial cancers) (43).

Anti PD-L1 therapy: Atezolizumab improve OS in the IMpower150 trial, as first-line treatment for metastatic NSCLC with no EGFR/ALK mutation when used in combination with standard chemotherapy than standard chemotherapy alone (44). Other trials with demonstrated superiority of PD-LI inhibitors and standard verse standard of care alone include the IMvigor210 trial for locally advance and metastatic urothelial cancers (45), Impassion-130 trial for triple negative breast cancer (46), IMpower133 for extensive stage small cell lung cancer (47). Avelumab demonstrated superiority in the JAVELIN trials (48) and Durvalumab in the PACIFIC trials (49).

Anti-LAG-3 therapy: Relatlimab in combination with Nivolumab showed an improved 12 months median progression free survival (47.7% vs 36%) in patients with previously untreated metastatic or unresectable melanoma when compared to Nivolumab monotherapy in the RELATIVITY-07 trail (50).

TABLE 2 Select cardiac pathology as a percentage of overall cardiac irAEs reported on Vigibase for each ICIs as of 2022*(VigiAccess, July 2022)*.

	Anti C TL4-A	Anti PD-1			Anti PD-L1				Anti-LAG-3
Select Cardiac irAEs	Ipilimu mab	Nivolu mab	Pembrolizu mab	Atezolizu mab	Aveluma b	Cemipli mab	Dostarlim ab	Durvalum ab	Relatlimab
Reporting timeline	2009-2002	2014-2022	2015-2022	2015-2022	2015-2022	2018-2022	2019-2022	2014-2022	2017-2022
Cardiac irAEs as percent of all irAEs (%)	2	2	2	2	3	3	5	3	6
Myocarditis (%)	22.7	20.7	19.9	15.8	20	27.3	14.2	21.4	33.3
Atrial Fibrillation (%)	16.3	11.2	9.7	15.4	14.4	7.6	7.1	11.6	n/a
Myocardial Infarction (%)	6.1	6.5	6.2	7.9	2.3	6.1	7.1	5.8	11.1
Cardiac arrest (%)	6.6	5.0	5.0	5.8	10.0	3.0	21.4	7.1	n.a
Cardiac Failure (%)	5.6	8.3	8.3	9.1	10.0	16.7	0.0	5.4	11.1

3 Cardiac irAEs of immune checkpoint inhibitors

Cardiac IrAEs have been reported in association with anti CTLA-4, anti PD-1, and their combinations. Reported cardiac toxicity is diverse, involving various cardiac tissues.

3.1 Epidemiology

The exact incidence of cardiac IrAEs resulting from ICI therapy have been difficult to quantify as early clinical trials testing efficacy of ICIs did not routinely evaluate for changes in cardiac function and myocardial injuries. Limited epidemiological data can be obtained from manufacturer safety databases, the World Health Organization (WHO) pharmacovigilance repository, (3) the FDA Adverse Event Reporting System (FAERS) database, and retrospective studies including meta-analysis of existing data and case reports. However, estimates from each source vary significantly. There is a possible underestimation of the incidence of cardiac irAEs for a host of reasons, ranging from the vagueness in its clinical presentation, the potential overlap with other cardiovascular disease and comorbidities, and a poor awareness of this condition (51). The WHO database reported higher incidence of ICI irAEs likely due to increased use of ICIs and improved recognition of their toxicities. Data from WHO database suggests myocarditis and arrhythmias as the most common cardiac irAEs. Table 2 shows selected cardiac morbidities as a percentage of overall cardiac irAEs reported on Vigibase for each ICI as of 2022. In a 2020 systematic review and meta-analysis, 0.1%-0.9% for myocarditis, 0.1%-1.0% for pericardial effusion, 0.0%-0.5% for cardiac failure, 0.3% for cardiomyopathy, 4.6% for atrial fibrillation, 0.0%-0.7% for myocardial infarction, and 0.1%-0.8% for cardiac arrest (52). Pharmacovigilance reporting systems may be limited by under-reporting, reporting bias, and a lack of information on population exposed to the drug. The risk associated with a drug is therefore difficult to quantify accurately in these databases (53–55).

Vigibase do not provide data on fatality. Wang and colleagues in a 2018 meta-analysis of 112 trials involving 19,217 patients showed toxicity-related fatality rates of 0.36% for anti-PD-1, 0.38% for anti-PD-L1, 1.08% for anti-CTLA-4), and 1.23% for combined anti-PD-1/PD-L1 plus CTLA-4 therapy (2). A 6 year (2011-2017) analysis of the Danish registry demonstrated an absolute risks for cardiac irAEs of (6.6–9.7%) with anti-PD1 and anti-CTL4 therapy, significantly higher than reports from pharmacovigilance studies (56). However, this study only included patients with malignant melanoma and lung malignancies which are generally considered high risk malignancies for irAEs. Moreover, the determination of what entails a cardiac irAE, which is not consistent between reports, may explain some discrepancy between various data repositories (57). Evidently, mortality is more frequent with combination PD-1/CTLA-4 blockade (58). There are currently no mortality data for anti-LAG-3 therapy and Vigibase cardiotoxicity data on Relatlimab should be characterized with caution due to a low sample of only 66 adverse events. Additional large prospective studies are needed to provide more precise estimates of the actual incidence and fatality rates of cardiotoxicity arising from ICI immunotherapy.

4 Mechanism of ICI induced cardiac IrAEs

The exact mechanism of ICI-associated cardiotoxicity is not yet fully understood (59). Proposed mechanisms include: (i) Direct destruction of cardiac tissue by deregulated, activated autoimmune T lymphocytes; (ii) Indirect destruction of cardiac structures by pro-inflammatory cytokines and other molecules

released by ICI deregulated T lymphocytes and the cells that they activate, such as macrophages; (iii) Recognition of cardiac self-antigens by autoantibodies to promote cell-mediated cardiotoxicity. These mechanisms can involve single or multiple cardiac structures resulting in pathologies.

4.1 Direct cellular destruction of cardiac tissue

Cardiac cells, like APCs and certain cancer cells, are now known to activate CTLA-4 and PD-1/PD-L1 pathways to maintain self-tolerance of cardiac structures during T lymphocyte responses to stress and stimulatory antigens under physiological conditions (60) (see Figure 2). CTLA-4 and PD-/PD-L1 blockade likely interrupt this immunologic homeostasis thereby causing auto-immune cardiac toxicity mediated by deregulated T-lymphocytes. Evidence for this theory stem from histological and immunohistochemical analyses demonstrating membrane and cytoplasmic expression of PD-L1 in injured cardiac tissue (61, 62). PD-L1 expression is higher in cardiac tissue samples from patients with ICI-associated myocarditis, which is consistent with lymphocytic myocarditis as histologically characterized by myocardial infiltration of macrophages and CD4+/CD8+ T lymphocytes (63, 64). In a preclinical study, Grabie et al. demonstrated the expression of PD-LI on cardiac endothelium which has a cardio-protective effect against T lymphocyte-mediated cardiac injury (65). Preclinical insights from genetic and manipulation of immune checkpoint pathway have further bolstered this theory. For example, PD-1 and CTLA-4 knockout mice develop rapid lymphoproliferation and fatal T cell mediated myocarditis (66).

Cellular infiltration of cardiac myocytes in irAEs may also be due to the immune polarization effects of ICIs (67) . For example, anti-PD1 has been found to transduce immunoregulatory signals that modulate macrophage polarization to pro-inflammatory phenotype *via* the inhibitory effects of microRNA-34a (miR-34a) on the Krüppel-like factor 4 (KLF4) signaling pathway. Consistent with this finding, among other activities, the transcription factor KLF4 has anti-inflammatory properties with a cardiac protective effect. Xia and colleagues hypothesize that miR-34a mediated inhibition of the KLF4 pathway leading to inflammatory macrophage activity may account for the cellular infiltration and destruction of cardiac tissues seen in ICI therapy. In their *in-vivo* experiment, anti-PD1 treatment was shown to induce polarization of pro-inflammatory macrophages accompanied by increased MicroRNA-34a expression and decreased expression of KLF4, resulting in cardiac injury 67).

4.2 Cardiac antigen immune reactivity

There is ample of evidence to suggest the existence of common T-cell receptors or epitopes between certain cardiac

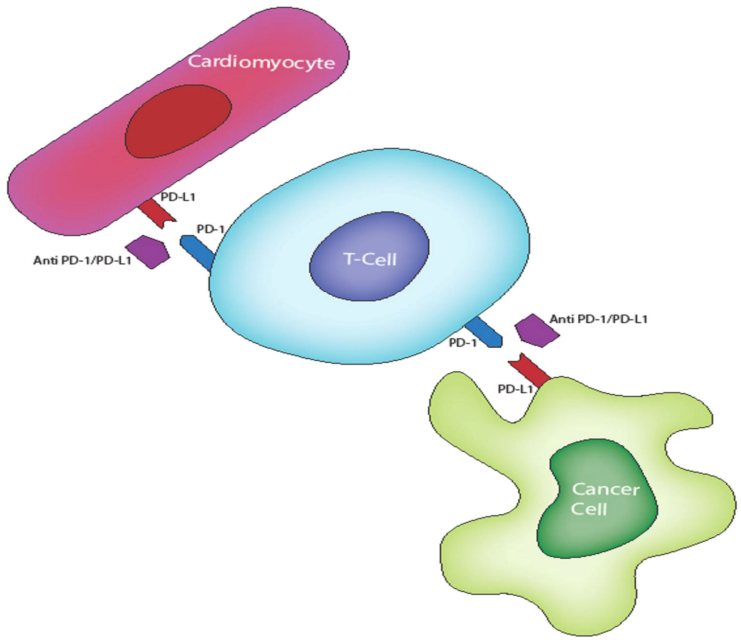

FIGURE 2
PO-L1 expression on cardiac tissues confers protection from activated T cell *via* P0-1/PO-L1 inhibition of T cells. This inhibition is lost in ICI therapy resulting in an autoimmune T lymphocyte destruction of cardiac tissues.

myocytes and tumor (68, 69). This shared antigen theory is supported by the relatively early onset of myocarditis observed after initiating ICI therapy in a select group of patients. It is quite possible that a pre-existing molecular mimicry that allows an immune evasion for these cardiac cells in a similar fashion to the tumors become disrupted, predisposing these patients to the development of myocarditis when treated with ICIs (69). However, multiple questions remain to be answered with respect to this hypothesis such as the nature of these epitopes, how they elicit an immune response, and how immune effectors are targeted to cardiac tissue. While these questions abound, recent translational studies suggest a second hit may be necessary to initiate cardiac immune reactivity (70). In a study by Michel and colleagues, mice models with transplanted tumors developed left ventricular (LV) dysfunction with the initiation of ICI therapy. In contrast, LV dysfunction was undetectable in tumor-free mice receiving the same ICI therapy. This finding has led to the postulation of a second hit theory, which argues that a form of systemic stress induced by the presence of the tumor may be required to initiate the cardiac immune reactivity in predisposed patient (70). In addition, anti- PD-1 therapy is now recognized to drive the development of auto-antibodies against cardiac specific proteins. Okazaki et al. demonstrated that mice deficient in PD-1 develop autoimmune dilated cardiomyopathy with production of high-titer autoantibodies against the cardiac-specific protein cardiac troponin I (cTnI) (71, 72). Further investigation demonstrates that the anti cTnl autoantibodies induces heart dysfunction and dilation through chronic stimulation of Ca^{2+} influx into cardiomyocytes (71, 72). Other auto-antibodies induced by ICI therapy with the potential to initiate or escalate cardiac irAEs include antibodies reactive with acetylcholine receptors, striated muscle cells, mitochondria, alanyl-tRNA synthetase, signal recognition particle (SRP), and 3-hydroxy-3-methylglutaryl-coenzyme A reductase (73–76). These auto-antibodies have been associated with myocarditis, primarily mediated through cross reactivity with cardiac striated muscle antigens and/or inducing antibody-dependent cellular cytotoxicity (ADCC) (73–76).

4.3 ICI induced cytokines release

The production of pro-inflammatory cytokines is upregulated by therapies that activate certain T cell subsets, leading to a constellation of non-specific inflammatory processes known as the cytokine release syndrome (CRS) (77, 78) (see Figure 3). In CRS, T cells, NK cells, APCs and endothelial cells, release a variety of cytokines at supraphysiologic levels (77) Interleukin-6 (IL-6) is most implicated in CRS (77). Other molecules associated with CRS include interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNFα); nitric oxide (NO); nitric oxide synthase (NOS); and reactive oxygen species (ROS) [18]. These cytokines and radicals can have cytotoxic effects on cardiac myocytes, resulting in arrhythmias, conduction abnormalities, impaired contractility, and other cardiac anomalies (78, 79). CRS is however less common with ICIs when compared with other novel cancer immunotherapeutic such as the chimeric antigen receptor (CAR) T cell therapy (80). Findings from Vigibase data on adverse drug reactions suggests CRS incidence to range from 0.05% to 0.14% for ICIs, and more common with anti-PD1/PD-L1 combination therapies (77).

4.4 Dysregulation of myocardial metabolism

Michel and colleagues propose a metabolic pathway leading to myocardial dysfunction due to anti-PD1 therapy based on substrate analysis in experimental model (see Figure 4) (70, 81) . Molecular analysis of cardiomyocytes from mice treated with anti-PD1 therapy shows metabolic disturbances including a reduction in metabolites such as carnitine/acylcarnitine carrier protein, acyl-CoA dehydrogenase, acyl-CoA synthetase pyruvate dehydrogenase kinase 4 (PDK-4), and pyruvate carboxylase with a concomitant increase in beta-oxidation substrates, cardiac TNF-alpha and 1,3-bisphosphoglyceric acid (70). These measures indicate changes in lipid and glucose metabolism capable of altering oxidative phosphorylation, mitochondrial function, plasma membrane permeability, and other cellular functions, ultimately leading to cell death. This dysregulation of myocardial metabolism seen with ICI therapy is likely to be a downstream effect of the immune/inflammatory pathologies caused by the cardiac irAE mechanisms already discussed above but may also drive currently underappreciated aspects of the disease process.

5 Clinical risk factors for ICI induced cardiac IrAEs

Identification of patients at risk for ICI induced IrAEs is difficult and an ongoing area of research. A risk predictive model is needed to provide a basis for the clinical use of ICIs, as well as a guide for the prompt management of ICI toxicities. Identified patient-related risk factors for cardiac IrAEs include pre-existing cardiovascular diseases, co-morbidities (such as hypertension and diabetes mellitus), age, sex, underlying autoimmune diseases, opportunistic pathogens, medications, tumor-related factors, and genetic predisposition. Therapy-related risk factors include the use of combinatorial cancer therapy (such as irradiation, chemotherapy, targeted therapies, and other ICIs or immunotherapies), specific ICIs and their dosage.

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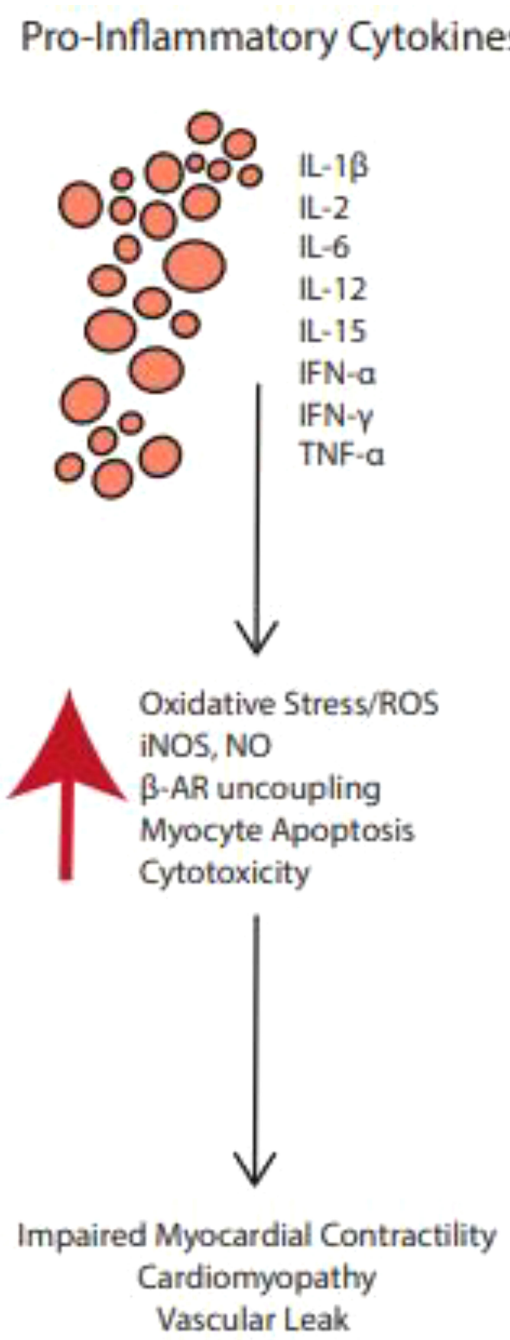

FIGURE 3
Pro-inflammatory cytokines upregulated by ICI therapies may activate certain T cell subsets, leading to a constellation of non-specific inflammatory processes known as the cytokine release syndrome.

5.1 Patient related risk factors

Several possible baseline risk factors proposed for IrAEs in general have little prospective evidence to support their association with the development of cardiac specific IrAEs. Females have been reported to be associated with higher rates of IrAEs although this phenomenon lacks mechanistic explanations (82). Age group and BMI as a risk factor have yielded conflicting reports in retrospective studies (82). One retrospective study demonstrated an association of ICI IrAEs

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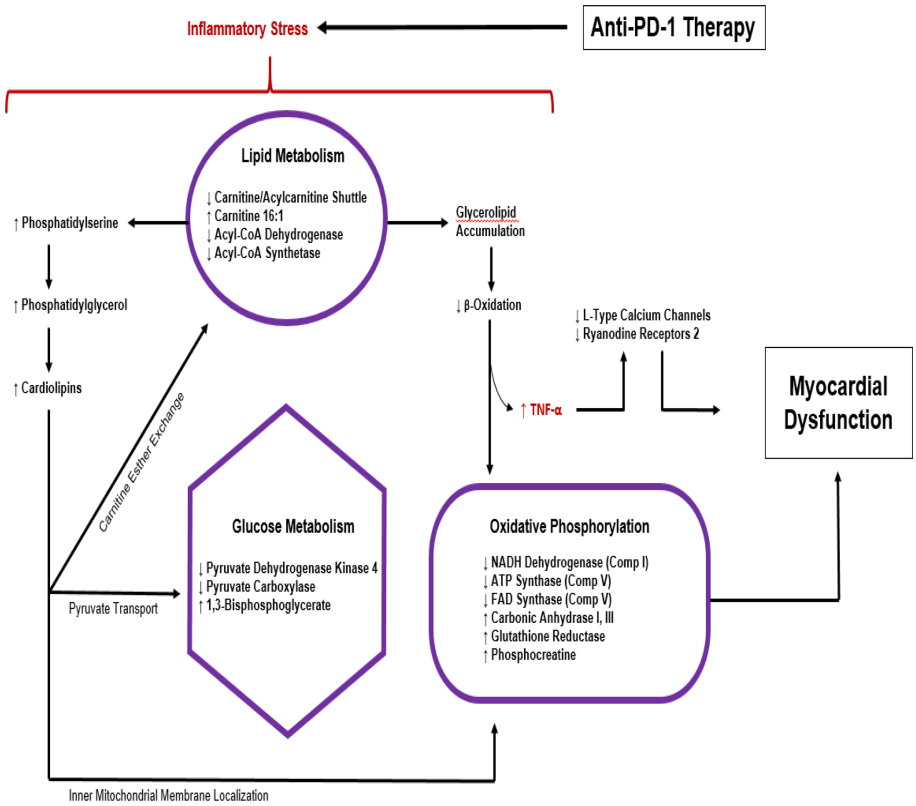

FIGURE 4
Metabolic response to anti-PD1 therapy based on substrate analysis in experimental model (70, 81).

with patient BMI. IrAEs were found to increase by 9% with every BMI increase by 1 kg/m^2 (83). The occurrence of certain toxicities varies depending on the type of malignancy and/or pathway blocked. Patients with lung cancer are notable for increased odds of irAEs or irAEs requiring hospital admission when compared to patients with other malignancies (melanoma OR (odd ratio): 0.70, renal cell carcinoma OR: 0.71, other malignancy OR: 0.50) (84). Hazard ratios of 2.14 (95% CI 1.50-3.05) in patients with lung cancer and 4.30 (1.38-13.42) and 4.93 (2.45-9.94) have been demonstrated in patients with malignant melanoma treated with anti-PD1 and anti-CTLA-4, respectively (56). Furthermore, a circulating neutrophil/lymphocyte ratio greater than 3.0 at the time of starting treatment has been correlated with a lower risk of IrAEs (83). Pre-existing auto-immune disorders may also increase risk for ICI IrAEs as reported in multiple case series (85). However, this remains unclear. Baseline cardiac pathologies is also a risk factor. In the Phase III Javelin Renal 101 trial of ICI and targeted therapy combination, patients with elevated baseline troponin suggestive of baseline cardiac pathologies and autoimmune diseases were shown to have higher risk of major cardiac irAEs when compared to patients with low baseline troponin values (86).

5.2 Therapy-related risk factors

5.2.1 Combinatorial therapy

ICI combinations, either with other ICIs or with other oncologic therapies such as chemotherapy, radiation therapy, and targeted therapy have significantly improved prognosis for many cancers. The cardiac irAEs of combination regimens involving ICIs and other conventional therapies is an active area of investigation as toxicities inherent to individual therapies may amplify with various combinations.

i. **Dual ICI therapy:** Clinical benefits of combination ICI have been demonstrated in multiple randomized clinical trials. However, this often comes at a cost of exacerbated treatment toxicities. In a recent database review of over 14,000 patients who received ICI in the United Sates, combination ICIs (anti-PD-1/PD-L1 and CTLA-4) were associated with a more than two fold increase in odds of developing IrAEs requiring hospital admission which were particularly noticeable in lung malignancies (84). In this study, incidence of irAEs warranting hospital admission was 3.3% for patients treated with anti-PD-1 antibodies, 1.1% for patients

TABLE 3 Current Pathology Grading criteria for ICI induced myocardial inflammation.

3a. Palaskas et.al Grading Criteria (117)

Grade	Pathologic features
0	Negative
1- Myocardial inflammation	Multifocal inflammatory infiltrates without overt cardiomyocytes loss by light microscopy
1A	Mild inflammatory cell score by immunohistochemistry (10-20 inflammatory cells/ high power field)
1B	At least moderate inflammatory cell score by immunohistochemistry (>20 inflammatory cells/ high power field)
2- Definite myocarditis	Multifocal inflammatory cell infiltrates (>40 inflammatory cells/ high power field)

3b. Champion and Stone Grading Criteria (118)

Grade	Immunohistochemistry
Low Grade	(50 CD3+ cells/high power field)
High Grade	>50 CD3+ cells/high power field

treated with anti-PD-L1 antibodies, 3.9% for patients receiving anti-CTLA-4 antibodies, and 3.5% overall for all ICI antibodies as monotherapy. However, hospitalization rates was increased to 7.3% for patients on combination therapy (84, 87). Hu in his systematic review and meta-analysis of 2,551 studies with 20,244 patients reported an increased risk of cardiac arrhythmia with ICI combination (anti PD-1 and anti CTLA-4) therapy compared to either agent as monotherapy (OR 3.90, 95% CI: 1.08–14.06, p = 0.603) (88). Also, WHO database reports mortality from ICI-associated myocarditis to have an almost two fold increase with combination ICIs (60% versus 36%) when compared to patients who receive anti-PD-(L)1 monotherapy (58).

ii. **Chemotherapy and Targeted therapies:** Many conventional chemotherapies unfortunately have cardiotoxicities effects that can be amplified with ICIs whether delivered concurrently or sequentially. The hypothesized mechanisms of chemotherapy-induced cardiotoxicity vary by agents. For example, anthracyclines may have direct cellular toxicity *via* mitochondrial damage, with cumulative myocardial injury, resulting in both diastolic and systolic dysfunction (89). Taxanes cause myocardial damage *via* their effects on subcellular organelles, or through the induction of massive histamine release, and are associated with conduction disturbances and arrhythmias (89). 5-Fluorouracil has direct toxic effects on the vascular endothelium which can cause spasm of coronary vessels, platelet aggregation, and thromboxane formation, increasing thrombogenesis and cardiac injuries. The potentiation of these chemotherapy-associated and/or ICI-associated cardiac IrAEs in chemotherapy-ICI combination therapy is an active area of research. Meta-analysis however, demonstrates an increase in cardiac IrAEs when a chemotherapeutic agent is combined with ICI [85]. In Hu's study, PD-1 blockade plus chemotherapy exhibited a significant increase in all grades of myocardial disease when compared with chemotherapy alone (88). Aside from the conventional systemic therapies, cardiac toxicity in targeted therapies is increasingly also being recognized. Trastuzumab (an anti-erbB2) for example is known to cause left ventricular dysfunction and the induction of congestive heart failure. BRAF and MEK inhibitors can also cause a decline in left ventricular ejection fraction (90). There is a demonstrable risk of myocardial infarction, atrial fibrillation, and QTc prolongation with BRAF inhibitor therapy which is theorized to be caused by a BRAF-mediated alteration of the myocardial repolarization process (91). The potentiation of cardiac toxicities due to treatment with ICIs in combination with these targeted therapeutics has yet to be explored in a clinical trial. However, insights from pre-clinical studies demonstrate a 3 fold increased calcium overload and reduced viability of human cardiomyocytes treated with the combination of Pembrolizumab and Trastuzumab compared to cells treated with either reagent alone (92, 93). The pembrolizumab-trastuzumab combination, when compared to monotherapy, was also noted to increase inflammation affecting cardiac cells and cardiac fibrosis by enhancing the expression of NF-kB and interleukins (93).

iii. **Radiation Therapy**: The effects of radiotherapy (RT) on both tumors and its microenvironment involves a complex manipulation of immune system. Radiotherapy has potential to alter the tumor immune microenvironment

TABLE 4 ASCO grading for ICI induced myocarditis is based on biomarkers, ECG, imaging and clinical presentation (120).

	Grade 1	Grade 2	Grade 3	Grade 4
Clinical symptoms	Asymptomatic	Mild	Moderate (Symptom with mild activity)	Moderate to severe decompensation, IV medication or intervention required, life-threatening conditions
Cardiac Biomarkers	Abnormal	Abnormal	Abnormal	Abnormal
ECG	Abnormal	Abnormal	Abnormal	Abnormal
TTE	–	–	LVEF <50% or regional wall motion	LVEF <50% or regional wall motion
Cardiac MRI	–	–	Cardiac MRI diagnostic or suggestive of myocarditis	Cardiac MRI diagnostic or suggestive of myocarditis

to augment the antitumor effects of ICIs, specifically by releasing cytokines, endogenous danger signals, increasing the presentation of tumor-associated antigens by APC, and stimulating diversification of the anti-tumor T cell repertoire (94). Wang and colleagues demonstrated RT and anti-PD1 synergy to improve clinical endpoints may result from RT overcoming PD-1 inhibitor resistance by inducing the production of type I interferon (IFN) leading to an enhancement of MHC class 1 expression (95, 95). Lee and colleague showed radiation therapy at an ablative dose can have an anti-tumor effects that are dependent on cytotoxic T-cells (96). Other studies have observed an abscopal effect where radiation therapy of primary tumor could have a potent effect on non-irradiated tumor cells (94) (94). However, combined radiotherapy and ICI may also affect both the type and severity of immune related toxicities, including cardiotoxicity. For example, the combination of thoracic radiation and PD-1 blockade can exacerbate radiation-initiated cardiac inflammation and cardiotoxicity (97, 98).

5.2.1 Dosage of ICI

The safety of ICIs given in combination with a variety of other cancer agents is clearly dependent on the dosage administered (99) . There is also evidence that this is the case for the risk of cardiac IrAEs (100). However, establishing safe doses for novel combination therapies involving ICIs has been challenging in face of the limited clinical experience with their utilization (99). In a meta-analysis by Bertrand and colleagues, the risk of developing all irAEs was dependent on dosage, with their incidence evaluated as 61% (95% CI, 56-66%) for ipilimumab at a dose of 3 mg/kg and 79% (95% CI, 69-89%) for ipilimumab at a dose 10 mg/kg (101). Another meta-analysis of 2,551 studies including 25 clinical trials and 20,244 patients treated for advanced melanoma show a decreased risk for all severe IrAEs with ipilimumab at 3 mg/kg every 3 weeks; pembrolizumab at 10 mg/kg every 2-3 weeks; and Nivolumab at 3 mg/kg every 2 weeks when compared with ipilimumab at 10 mg/kg every 3 weeks (102). The irAEs were unspecified in this study (102). Hu's cardiac specific meta-analysis however did not show any significant difference in cardiac IrAEs between ipilimumab at a dose of (3 mg/kg q3w) versus (10 mg/kg q3w) (88). Nivolumab at 3 mg/kg plus ipilimumab at 1 mg/kg also showed no increased risks of coronary artery disease compared with a dose of ipilimumab at 3 mg/kg plus Nivolumab at 1 mg/kg. Similarly, compared with a dose of 10 mg/kg q2w, a dose of 10mg/kg q3w PD-1 inhibitor (pembrolizumab) did not show significantly increase risks of cardiac failure (88). Dosage for a combination including ICI and a different kind of immunotherapeutic (such as CART-T) or a biologic agent is much more complex and requires additional study at this time (99).

6 Clinical diagnosis and management

6.1 Clinical manifestation

One main prerequisite for managing cardiac IrAEs is the knowledge and awareness of this complication. Subtle signs and symptoms which may become progressive need to be adequately interpreted to initiate management and avert complications.

Manifestations of cardiac irAEs range from a subclinical rise in cardiac biomarker and vague symptoms such as malaise to overt symptoms of chest pain, dyspnea, palpitations, progressive fatigue, pre-syncope and syncope that can lead to multiorgan failure, cardiogenic shock, and cardiac arrest. (59, 103). These symptoms may be obscured by other non-cardiac irAEs such as myositis, hypothyroidism, pneumonitis, or other symptoms related to the primary malignancy or comorbid conditions. The median time to onset of clinical manifestation of cardiac irAEs is 6 weeks (typically 3 to 9 weeks) but can range from 2 to 54 weeks (104), typically corresponding to the period after the first and third infusion (105). The average time until symptoms vary for each ICI type, cancer type, type of cardiotoxicity, and delivery with other therapeutics (104). On literature review, the anti-PD-L1 ICIs were found to have an earlier median time to presentation of symptoms (1-9 weeks for Atezolizumab and Durvalumab) (104). The anti-CTLA-A agent ipilimumab had a longer median onset time of 10 weeks, however in combination with nivolumab this median time was reduced to 6 weeks (104).

6.2 Clinical investigation

A detailed history, review of systems, and physical exams is required to exclude other cardiac diseases. Blood tests, electrocardiograms (ECGs), chest X-ray, and trans-thoracic echocardiograms (TTEs) are needed for diagnosis and management. Laboratory tests typically include the assessment of serum levels of cardiac troponins (including cardiac troponin I [cTnI] and troponin T [cTnT]), creatine phosphokinase (CPK), creatine kinase (CK), and creatine kinase-myocardial band (CK-MB). Others include brain natriuretic peptide (BNP), and N-terminal pro-brain natriuretic peptide (NT-proBNP) (59, 103). Additional testing such as stress tests, cardiac catheterization, and cardiac MRI may be guided by the cardiologist (103).

6.2.1 Laboratory investigations

A hallmark of ICI induced myocarditis is an increase in serum cardiac biomarkers, notably troponin, BNP, NT-proBNP, and CK-MB which are further discussed in the biomarker subsection of this review.

6.2.2 Electrocardiography

ECG is often a first-line test to identify patients with suspected cardiac irAEs. A 12-lead ECG should immediately be performed once a patient complains of chest pain, palpitations, dizziness, dyspnea, or any other concerning cardiac symptom (106). Abnormal ECGs have been reported in 40–89% of patients with ICI related toxicities. ECG abnormalities that may raise suspicion of cardiotoxicity include abnormal PR interval, ST-segment depression and elevation, atrioventricular block, ventricular arrhythmias, T-wave inversions, and new Q waves anomalies (107), (103, 106). T wave changes are the most common ECG abnormalities seen ECG changes in ICI cardiac events (107), (103, 106). ECG should be carefully interpreted with context to the patient as anomalies are common in the cancer patient population which do not always indicate a cardiac irAEs. Collecting a baseline ECG allows for recognition of any change occurring during ICI therapy, facilitating early diagnosis of associated cardiotoxicity (106).

6.2.3 Cardiac imaging

For concerns of an acute coronary syndrome, emergency coronary angiography may be indicated for patients presenting with abnormal cardiac biomarkers and ECG or ischemic symptoms. In addition, TTE could provide further insight into motion anomalies of the myocardium and left ventricular ejection fraction (LVEF) compromise. TTE anomalies may be detected at later stage of ICI-associated myocarditis. Cardiac MRI (CMR) has a diagnostic superiority to TTE because it can identify fibrosis and inflammation tissue characteristics in the early course of the disease. ICI myocarditis is typically defined using the modified Lake Louise Criteria (108, 109). An analysis of clinical, CMR, and histopathological findings of patients on ICIs from international registries and retrospective studies shows that T1 mapping and application of the modified Lake Louise I or the updated Lake Louise II criteria provides important diagnostic value and prognostic value in patients with ICI-associated myocarditis (108, 110, 111). CMR and echocardiographic findings of impaired global circumferential strain, global radial strain, and global longitudinal strain in patients with an ICI associated myocarditis have been reported by many studies (112, 113). Other studies also showed a low sensitivity of CMR in detecting cardiac irAEs with features such as septal late gadolinium enhancement (LGE) seen only in 48% of patients (114). More also, LGEs result from the changes contrast uptake and washout patterns within the extracellular space could be seen in most myocardial injuries and therefore not specific for ICI-associated myocarditis (115). Further studies are needed to characterize cardiac MRI criteria for ICI-associated toxicities.

6.2.4 Endomyocardial biopsy

Endomyocardial biopsy (EMB) which is gold standard for the diagnosis of ICI myocarditis, should be considered for patients with concerns for myocarditis based on cardiac imaging, cardiovascularly unstable patients, and patients who fail to respond to initial treatment with steroids. EMB could also aid definitive diagnosis when diagnosis is in doubt. Myocardial features identified on EMB for ICI-associated myocarditis include interstitial fibrosis, lymphocyte infiltration, T cells

($CD4^+$, $CD8^+$), macrophage infiltration, and other inflammatory changes (116). Palaskas and colleagues recently developed a grading system for ICI myocarditis and myocardial inflammation by pathology findings on EMB and noted a correlation with clinical outcomes (see Table 3A) (117). Interestingly, the Palaskas et al. study identified patients with EMB confirmed grade 1 ICI induced myocarditis as a low-risk group that may be capable of continuing ICI therapy without immunomodulation (117). This finding is however difficult to routinely introduce to clinical practice give the need for an EMB for grading ICI-related myocarditis. Champion and Stone used EMB to classify ICI-associated myocarditis based on inflammatory cell accumulation in cardiac tissues into high-grade (>50 CD3+ cells/high power field) and low-grade (≤50 CD3+ cells/high power field) groups by EMB finding (118) (see Table 3B). High-grade patients had a fulminant clinical disease course leading to a hundred percent fatality, while patients with low-grade cell accumulation had a more indolent clinical course with a hundred percent overall survival (118). These findings illustrate the value of EMB assessment of the extent of inflammatory changes in cardiac tissue following ICI but standardized criteria are yet to be adopted for the histopathologic grading of ICI myocarditis (119) .

6.3 Treatment of ICI-induced cardiac irAEs

Treatment of ICI cardiac irAEs requires collaboration between the oncologist and cardiologist. In all cases, empirical treatment for ICI cardiotoxicity should be started once the suspicion is high, even before confirmatory pathologic testing is obtained. The 2021 American Society of Clinical Oncology Clinical (ASCO) practice guidelines recommends holding ICI therapy starting with Grade 1 cardiac irAEs and a permanent discontinuation of therapy for Grade 2 or higher toxicities (see Table 4) (103). ASCO guidelines also recommend that all-grade toxicities have early administration of high-dose corticosteroids, typically 1-2 mg/kg of prednisone oral or intravenous depending on symptoms (103).

An immediate transfer to a coronary care unit is recommended for patients with elevated troponin or conduction abnormalities (103, 120). Patients with no immediate response to low dose steroid (1-2mg/kg) may receive high dose steroid (1 g daily intravenous methylprednisolone) with addition of other immunosuppressive therapy such as mycophenolate, infliximab, or anti-thymocyte globulin (103). ASCO clinical practice guidelines recommend a steroid taper of at least 4 to 6 weeks. Aggressive initial steroid strategy is also an option (500-1000 mg daily), especially in clinically unstable patients (4, 121). Mahmood et al retrospectively compared high dose versus low-dose glucocorticoids and reports lower adverse events in patients who received high-dose steroids (4, 121). Although selection criteria for high-dose versus low-dose steroids were unclear in this retrospective series, the authors recommend pulse dose steroids at 1000 mg daily, followed by 1 mg/kg daily of either oral or intravenous steroids (4, 121).

In steroid refractory cases, alemtuzumab, infliximab, tocilizumab, or rituximab and the CTLA4 agonist (abatacept) can be considered. Caution is needed with use of infliximab as it has been associated with heart failure and is contraindicated at high doses in patients with moderate to severe heart failure. Plasmapheresis has also been used, with the goal of accelerating removal of the contributing drug (as well as any potential circulating autoantibodies). This approach is important with ICIs because their half-lives are extremely long: 14.5 days for Ipilimumab, 25.0 days for pembrolizumab, 26 to 27 days for Nivolumab and 27.0 days for Atezolizumab. Supportive management can entail inotropic therapy and even mechanical circulatory support, including extracorporeal membrane oxygenation, as a bridge to recovery, as has been shown in patients who developed fulminant myocarditis with cyclophosphamide and ICIs. Current treatment recommendations are notably based on anecdotal evidence and the life-threatening nature of cardiac complications.

7 Biomarkers

Molecular biomarkers are needed to predict which patients will experience cardiac IrAEs from ICI therapy. Several biomarkers such as the expression of programmed cell death ligand 1 (PD-L1), tumor mutation burden (TMB), and microsatellite instability-high (MSI-H)/mismatch repair-deficiency (dMMR) have proven to be predictors for anti-tumor efficacy of ICIs (122, 123). However there remains a pressing clinical need for the identification of biomarkers that can predict toxicities as well as help filter out the patients who may benefit most from these costly therapies from those at risk of major cardiac toxicities. There are few reports of biomarkers for the prediction of, or early detection of IrAEs in general. These include changes in the expression of cytokines/chemokines, cellular markers, autoantibodies, and genes. There is unfortunately no report describing markers selective for cardiac specific IrAEs (124). Currently, putative biomarkers for cardiac-specific IrAEs are limited to the serum levels of proteins such as cardiac troponin (cTn), and myoglobin but these are largely not selective for ICI IrAEs and not supported by extensive clinical validation.

7.1 Non cardiac biomarkers

Peripheral blood count (PBC): The indices and absolute values of peripheral blood components such as leukocytes, neutrophils and lymphocytes and platelets have been well established as prognostic markers for ICI responses and

outcomes in several cancers (125). Several studies have also demonstrated PBC indices as predictive of ICI toxicities. For example, a recent retrospective study showed that an absolute lymphocyte count >820 at 2 weeks following nivolumab initiation predicts the early onset of irAEs during in a 6-week study period (126). Routinely available absolute lymphocyte count may therefore be useful for identifying patients at risk of early onset of ICI irAEs (126). Prospective studies are warranted in this area.

Cytokines/Chemokines: Lim and colleagues recently profiled the expression of 65 cytokines in 98 patients with melanoma treated with PD-1 inhibitors alone or in combination with anti-CTLA-4 (127). Cytokine expression was found to strongly correlate with irAEs warranting discontinuation of treatment and administration of high-dose steroids. Eleven cytokines significantly upregulated in patients with severe irAEs were integrated into a single toxicity score known as the CYTOX (cytokine toxicity) score. The most predictive cytokines for ICI toxicities include G-CSF, GM-CSF, Fractalkine, FGF-2, IFN-aplha2, IL12p70, IL1a, IL1B, IL1RA, IL2, and IL13 (127). The predictive utility of CYTOX score was confirmed in an independent validation cohort of 49 patients treated with combination anti-PD-1 and anti-CTLA-4 (127). The utility of CYTOX in predicting cardiac specific IrAEs has yet to be validated.

7.2 Cardiac specific biomarkers

Cardiac Troponins: Cardiac troponin T (cTnT) and cardiac troponin I (cTnI) are expressed exclusively in the myocardium. They are elevated in 84% to 94% of patients with ICI cardiotoxicity (including subclinical toxicities) (59, 105, 121). cTnI is often preferred for cardiac IrAEs as cTnT and other cardiac biomarkers such as CPK, BNP and/or proBNP may be elevated in patients with concurrent pathologies associated with ICI. For example, CPK is elevated in myositis which can be immune mediated. (cTn) are released after cardiomyocytes damage induced by various mechanisms such as ischemia, inflammation, oxidative stress, or apoptosis. Several studies have reported increased risk of ICI induced cardiac irAEs in patients with elevated pre-treatment troponin. Mahmood and colleagues compared the data of patients with and without myocarditis after ICI treatment and found a four-fold increase in the risk of cardiac irAEs for patients with troponin T (cTnT) ≥ 1.5 ng/ml (116, 121, 128). Another retrospective cohort study demonstrated a seven fold risk of cardiac IrAEs in patient receiving ICIs with baseline troponin >0.01 ng/ml (HR: 7.27; 95% CI: 2.72 to 19.43; $p < 0.001$) (129). Although currently not recommended by the ASCO updated guideline, there is a growing consensus to perform baseline troponin measurements prior to initiating ICIs The Heart Failure Association Cardio-Oncology Study Group and the International Cardio-Oncology Society risk stratification guidelines for anticancer therapies recommends pretreatment troponin determination (130).

Cardiac Auto-antibodies: Okazaki and colleagues showed that dilated cardiomyopathy in PD-1 deficient mice is associated with their production of high titer autoantibody against cardiac troponin I (71, 72). Cardiac troponin I auto antibodies have yet to be validated as a biomarker for cardiac irAEs.

Brain-type natriuretic peptide (BNP): BNP and N-terminal pro-brain natriuretic peptide (NT-proBNP) are standard biomarkers used in clinical practice for the diagnosis and management of heart failure. However, conclusions regarding the role of natriuretic peptides for the risk analysis and diagnosis of ICI cardiotoxicity remain undefined. A retrospective studies demonstrates an increased risk of ICI adverse event at B-type natriuretic peptide (BNP) >100 pg/ml (HR: 2.65; 95% CI: 1.01 to 6.92; $p = 0.047$) (129).

8 Roadmap to overcoming ICI-induced cardiac irAEs

8.1 Development and validating of prognostic biomarkers for cardiac irAEs

As discussed, existing biomarkers for ICI cardiac irAEs have relatively limited clinical data and/or lack extensive validation. Biomarkers that are appropriately sensitive and specific to therapy-induced injury could find applications in long-term post therapy management, subclinical toxicity detection, and pre therapy risk stratification for ICI therapy (131). Future biomarkers for cardiac irAEs would be sensitive enough to detect subclinical conditions but specific enough not to arise from the cancer itself. Several have been proposed or are under investigation. Modern capabilities in systems biology and genetics have enabled novel techniques like high-through sensitive bioassays and multiomics approaches (131). Currently proposed blood biomarkers include high-sensitivity troponin levels (hs-TnI), microRNAs, C-reactive protein, myeloperoxidase, galectin 3, interleukin family molecules including ST2, matrix metalloproteinase, placental growth factor (PlGF), growth differentiation factor 15, peripheral blood mononuclear cell gene expression profile, and human heart-type fatty acid-binding protein (132) (133). Many of these biomarkers are nonspecific to ICI as they have been detected at elevated levels following other systemic therapies and cardiac radiotherapy (133, 134). Nevertheless, pre-treatment hs-TnI levels (detected using a modification of the fourth-generation cTnT assay) at a cut-off of 14ng/L have been demonstrated to predict cardiovascular endpoints and the progression of cardiac involvement in patients receiving Nivolumab (135). It is notable in this regard that the Stanford Cancer Institute has recently implemented surveillance for ICI-associated myocarditis with hs-TnI assay (136, 137). Another predictive measure for cardiac irAEs severity following ICI therapy may be the levels of certain microRNAs. Pre-clinical studies have demonstrated an increased frequency and severity of irAEs in murine models deficient in miR-146a and studies of

humans subjects have demonstrated an increased risk of severe irAEs in patients on anti PD-1 therapy who have a single nucleotide polymorphism (SNP) in miR-146a (138). MiR-34a is a critical regulator of myocardial physiology that increases with age and has been associated with cardiac senescence and dysfunction. Through a variety of effects on the NF-κB and KLF4 signaling pathways miR-34a also modulates T cell and macrophage functions such that elevated levels may predispose patients to ICI-related cardiac toxicities (67, 139–141). Further studies of baseline and post-treatment levels of these and other miRs are required to substantiate the likelihood that these may have utility as prognostic biomarkers for ICI cardiac irAEs.

Besides circulating biomarkers, functional and MRI imaging markers have also been proposed to predict ICI toxicities. Cardiac PET scans entail exposure to ionizing radiation, but studies suggest they may be indicated for measuring long-term ICI effects on the heart (142) . Advanced radioscopic imaging techniques may also evaluate myocardial and vascular changes at the molecular level (142). A recent retrospective study identified septal late gadolinium enhancement as a possible predictor of cardiac event in patients receiving ICIs (143). It will be essential to contextualize any findings from circulatory and imaging biomarkers with the specific mechanism of IrAEs. For example, ICI-associated myocarditis biomarkers may detect between the different phenotypes of myocarditis; lymphocytic myocarditis is facilitated by proinflammatory T_H17 cells and CCR5, and giant cell myocarditis is thought to originate from the autoantigen-triggered immunoproteasome, leading to $CD4^+$ T cell recruitment and differentiation into T_H1 and T_H17 cells (64). Specific biomarkers along these immunological axes may be candidates for novel biomarkers of ICI-specific cardiac irAEs.

8.2 Utilization of immune checkpoint inhibitors with reduced cardiotoxicity

A shift in focus to research and development of novel ICIs which target antigens that are not shared amongst both the myocardium and tumor in question, unlike the current targets PD-1, PD-L1, CTLA-4, and LAG-3 may limit inflammatory reactions against cardiomyocytes. New drugs under investigation include anti-TIM-3 (T cell immunoglobulin and mucin-containing protein 3), anti-VISTA (V-domain Ig suppressor of T cell activation), anti-TIGIT, and anti-BTLA antibodies (144). These targets have each been shown to restore antitumor immunologic response in preclinical studies, and they are currently under study in humans (144). Cardiotoxicity of these agent are currently unknown. It is of utmost importance that these ongoing human studies prioritize the assessment of adverse event including cardiac toxicities in addition to cancer outcomes

8.3 Novel prophylaxis and therapies for cardiac irAEs

Current strategy for management of for ICI induced irAEs are empirical as no studies have specifically addressed the issue. There is potential for further development of anti-inflammatory agents that are specific to the myocardium, which may be administered prophylactically or in combination with current ICIs to avert cardiac irAEs. Immune modulators which have been shown in case reports or small case series to be effective in reversing near-lethal ICI-myocarditis. Drugs which have been investigated include tocilizumab (IL-6R antibody) (145), alemtuzumab (anti-CD52) (17) (146), abatacept (CTLA-4 agonist) (147), ruxolitinib (JAK inhibitor) (148), infliximab (TNFα antibody) (149), tofacitinib (JAK inhibitor) (150), mycophenolate mofitil (151), and antithymocyte globulin (152), and IV immunoglobulin. (153) However, the effectiveness of these therapies in ICI induced cardiac irAEs is unclear and they are therefore only reserved for patients with poor responses to corticosteroids. Further studies are needed to better understand the clinical indication and safe dosage for these drugs in patients with cardiac irAEs (154). For example, the ongoing ATRIUM trial (Clinicaltrial.gov NCT05335928) is being carried out to assess whether abatacept therapy, as compared to placebo, is associated with a reduction in major adverse cardiac events (MACE) among participants hospitalized for ICI-induced myocarditis.

The recent findings that anti-PD-1 therapy induces metabolic dysregulation associated with cardiac dysfunction raises the prospect of metabolic intervention for cardiac irAEs (70, 155) (81). Increased expression of TNFα is a notable downstream effect of anti-PD1 therapy which can lead to myocardial dysfunction *via* suppression of L-type calcium channel and ryanodine receptor-2 activities in addition to its pro-inflammatory activities. Michel and colleagues demonstrated that TNFα blockade could avert the associated subclinical manifestation of cardiac dysfunction due to anti-PD1 therapy in mice models without attenuating its anti-cancer efficacy. They hypothesize TNFα blockade may serve as a novel cardioprotective treatment against ICI therapy (70, 81, 155, 156) . Such an outcome may be expected as inflammatory mechanisms driven by TNFα are likely to have responsibility for ICI-induced cardiotoxicity but be less important for T cell-mediated anti-tumor immunity.

9 Conclusion

In conclusion, some advances have been made in elucidating the pathologic mechanisms of ICI-associated cardiac irAEs in recent years. Histopathologic grading

criteria with diagnostic and prognostic values have been developed but are yet to be standardized and universally adopted. Potential strategies for mitigating ICI-associated irAEs include: Developing and validating predictive biomarkers; developing and utilizing less cardiotoxic ICIs; administering prophylactically or in combination with ICIs to avert cardiac irAEs; and prospective trials of known anti-inflammatory agents with therapeutic benefit in patients with cardiac irAEs.

Author contributions

OI researched data for the article, made substantial contributions to discussions of the content and wrote the article. NN, YS, MN, and SP made substantial contribution to the writing and illustration of figures. ES, DS, DH, and BL made substantial contributions to discussions of the content and reviewed and/or edited the manuscript before submission. All authors contributed to the article and approved the submitted version.

Funding

BL National Cancer Institute NCI 1R01 CA252484-01.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 11 October 2022
DOI 10.3389/fonc.2022.1007577

Check for updates

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Radiation Oncology, a section of the journal Frontiers in Oncology

RECEIVED 30 July 2022
ACCEPTED 16 September 2022
PUBLISHED 11 October 2022

CITATION
Banfill K, Abravan A, van Herk M, Sun F, Franks K, McWilliam A and Faivre-Finn C (2022) Heart dose and cardiac comorbidities influence death with a cardiac cause following hypofractionated radiotherapy for lung cancer. *Front. Oncol.* 12:1007577. doi: 10.3389/fonc.2022.1007577

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Heart dose and cardiac comorbidities influence death with a cardiac cause following hypofractionated radiotherapy for lung cancer

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Background: There is increasing evidence of cardiac toxicity of thoracic radiotherapy however, it is difficult to draw conclusions on cardiac dose constraints due to the heterogeneity of published studies. Moreover, few studies record data on cause of death. The aim of this paper is to investigate the relationship between conventional cardiac dosimetric parameters and death with cardiac causes using data from the UK national cause of death registry.

Methods: Data on cancer diagnosis, treatment and cause of death following radical lung cancer radiotherapy were obtained from Public Health England for all patients treated at the Christie NHS Foundation Trust between 1/1/10 and 31/12/16. Individuals with metastatic disease and those who received multiple courses of thoracic radiotherapy where excluded. All patients who received > 45Gy in 20 fractions were included. Cardiac cause of death was defined as the following ICD-10 codes on death certificate: I20-I25; I30-I32; I34-I37; I40-I52. Cardiac V5Gy, V30Gy, V50Gy and mean heart dose (MHD) were extracted. Cumulative incidence of death with cardiac causes were plotted for each cardiac dosimetric parameter. Multi-variable Fine and Gray competing risk analysis was used to model predictors for cardiac death with non-cardiac death as a competing risk.

Results: Cardiac dosimetric parameters were available for 967 individuals, 110 died with a cardiac cause (11.4%). Patients with a cardiac comorbidity had an increased risk of death with a cardiac cause compared with those without a cardiac comorbidity (2-year cumulative incidence 21.3% v 6.2%, p<0.001). In patients with a pre-existing cardiac comorbidity, heart V30Gy ≥ 15% was associated with higher cumulative incidence of death with a cardiac cause compared to patients with heart V30Gy <15% (2-year rate 25.8% v 17.3%, p=0.05). In patients without a cardiac comorbidity, after adjusting for tumour and cardiac risk factors, MHD (aHR 1.07, 1.01-1.13, p=0.021), heart V5Gy (aHR 1.01, 1-1.13, p=0.05) and heart V30Gy (aHR 1.04, 1-1.07, p=0.039) were associated with cardiac death.

10.3389/fonc.2022.1007577

Conclusion: The effect of cardiac radiation dose on cardiac-related death following thoracic radiotherapy is different in patients with and without cardiac comorbidities. Therefore patients' cardiovascular risk factors should be identified and managed alongside radiotherapy for lung cancer.

KEYWORDS

lung cancer, radiotherapy, cardiac toxicity, dose constraint, cardiac comorbidities

Introduction

Lung cancer is the third most commonly diagnosed cancer in the UK and radiotherapy is used as the primary treatment for lung cancer in 20-55% of patients (1). One third of patients treated for lung cancer will have concomitant cardiovascular disease or have cardiovascular risk factors such as hypertension or diabetes (2–4).

Improvements in radiotherapy technology over the last 2 decades, including image guided radiotherapy (IGRT) and intensity modulated radiotherapy (IMRT), have led to more conformal radiotherapy, allowing the treatment of patients with larger tumours and better avoidance of organs at risk. The increase in radiotherapy conformality has facilitated the delivery of ablative doses for early-stage lung cancer showing high rates of local control and comparable outcomes to surgical resection in some patient groups (5, 6). The same success has not been achieved with increasing radiotherapy dose in patients with stage III lung cancer where the seminal RTOG 0617 trial of radiotherapy dose escalation failed to demonstrate a survival benefit compared to standard dose (7). The RTOG 0617 trial highlighted the heart as an organ at risk (OAR) in thoracic radiotherapy and reported that the volume of heart receiving ≥ 5Gy (V5Gy) or ≥30Gy (V30Gy) were associated with worse overall survival (7, 8). Following RTOG 0617, there have been many retrospective, single institution studies on cardiac toxicity in lung cancer (9, 10). The most common whole heart dose parameters examined and found to be significantly associated with overall survival are mean heart dose (MHD), heart V5Gy and heart V30Gy (9). The majority of the studies reported so far on cardiac toxicity have the end point of overall survival (8, 11), cardiac events (12–14), or non-cancer death (15). Most patients in these studies were treated with concurrent chemoradiotherapy in daily fractions of 1.8-2Gy.

It is clear that there is uncertainty in the cardiac dose parameters to be used in radiotherapy planning, the interaction with cardiac comorbidities and their association with subsequent cardiac events and survival. Furthermore, there is a paucity of data in patients treated with hypofractionated radiotherapy which is increasingly used worldwide for the treatment of patients with lung cancer. Therefore, the primary aim of our study is to examine if commonly used whole heart dose parameters (MHD, V5Gy, V30Gy and V50Gy) predict for death with a cardiac cause in a large cohort of patients with lung cancer treated with hypofractionated radiotherapy.

Methods

Databases

The National Cancer Registration and Analysis Service (NCRAS) is a longitudinal cancer registry that collects data on all people living in England who are diagnosed with cancer. Data on every primary tumour are collected from 162 National Health Service Providers and include data on: staging, pathology, systemic treatment, radiotherapy and hospital activity. NCRAS can be linked with cause of death data supplied by the Office for National Statistics (ONS) and Hospital Episode Statistics (HES) data on all admissions to NHS hospitals in England. Data are coded using the International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10) (10). In addition, data on deprivation is available from NCRAS using the Index of Multiple Deprivation (IMD) which is a measure of relative deprivation for small, fixed geographic areas of the UK. IMD classifies these areas into five quintiles based on relative disadvantage, with quintile 1 being the least deprived and quintile 5 being the most deprived.

Approval was granted to collect and analyse patient data for this study by the Leeds East Research Ethics Committee (18/YH/0058) and the UK Computer Aided Theragnostics (UKCAT) Research Database Management Committee (REC reference: 17/NW/0060). The UKCAT project is based at The Christie NHS Foundation Trust and automatically collects, pseudonymises and stores data from the trust's electronic health record (16). Cohorts from both institutions were combined and linked with the national databases discussed above.

Study population

All patients with stage I to IV lung cancer treated with radical radiotherapy at The Christie NHS Foundation Trust between 1/1/2010 and 31/12/2016 were identified using UKCAT. Patients were de-anonymised and the NHS numbers used to link with NCRAS data, and then pseudonymised again prior to analysis.

Data on patient sex, Eastern Co-operative Group performance status (PS), smoking status and radiotherapy were obtained from the UKCAT database. Tumour stage and histology, systemic treatment, (IMD quintile) and cause of death were obtained from NCRAS. Tumour stage was based on the 2010 Union for International Cancer Control TNM Classification of lung tumours version 7. Histology was based on the International Classification of Diseases for Oncology Version 3 (ICD-O3). If data on tumour stage or histology were missing from the NCRAS dataset, then the UKCAT dataset was used. If NCRAS coded the patient as having diagnosis based on imaging and no histology was recorded, this was considered a clinical diagnosis.

The HES dataset was used to identify patients who had a cardiac comorbidity recorded during a hospital admission from 1/1/2010 to 31/12/2016; defined using the following ICD-10 codes: I11; I13; I20-I25; I30-I52 (Table 1). A patient was defined as dying with a cardiac cause if the following ICD-10 codes were present in part 1 or part 2 of the medical certificate of cause of death (MCCD): I20-I25; I30-I32; I34-I37; I40-I52 (Table 1). Death certificates were available for patients who died prior to 30th November 2017, therefore follow-up was censored at this date.

Patients were included in the study if they were treated with hypofractionated radiotherapy to the lungs, defined as a dose >45Gy delivered in 20 fractions. Patients who received more than one course of thoracic radiotherapy and those with stage M1b disease were excluded.

Radiotherapy planning and treatment

A 3-dimensional (3D) or 4-dimensional (4D) planning CT scan was carried out. In patients planned with 3D CT the gross tumour volume (GTV) was contoured and a 5mm margin was added for clinical target volume (CTV). The planning target volume was created by adding a 13mm sup/inf margin and 8mm margin radially to the CTV. In patients planned with a 4D CT, a motion adapted GTV was contoured using the maximum intensity projection and a 5mm margin added to create an internal target volume (ITV). A further 5mm isotropic margin was created from the ITV to create the planning target volume (PTV).

The heart was contoured to include the full extent of the pericardium from the superior aspect of the left pulmonary artery to the inferior aspect of the heart as described in the UK Stereotactic Ablative Radiotherapy (SABR) consortium guidance document (17). Cardiac contours were reviewed by one clinician (KB) and plans with cardiac contours that did not meet the guidelines were excluded from analysis.

Heart dose parameters of V30Gy < 40% and V40<30% were introduced during plan optimisation for this cohort in 2015. Other dose constraints used as part of the radiotherapy planning process include: spinal cord maximum dose < 44Gy, whole lungs-PTV V20Gy < 35% and lung-ITV (4D CT planning) or CTV (3D CT planning) mean dose < 20Gy. The following heart dose parameters were extracted from the radiotherapy planning data for patients with a validated heart contour: V5Gy, V30Gy, V50Gy and MHD.

Statistical analysis

Key baseline characteristics were summarised based on history of cardiac comorbidities. Categorical variables were compared using a Chi-squared test and continuous variables using a Mann-Whitney Utest. We conducted Cox regression for all-cause mortality to assess overall survival differences for commonly used whole heart dose constraints: MHD, V5Gy, V30Gy and V50Gy. Plots of Schoenfeld residuals were performed to check for proportional hazards assumptions. We calculated cumulative incidence estimates of death with a cardiac cause with a cutpoint of 10Gy MHD based on previous publications (12–14). For cardiac V5Gy, V30Gy and V50Gy the median value was used as the cutpoint for calculating cumulative incidence estimates as previous literature has not defined a threshold value. The relationship between cardiac dose parameters and death with a cardiac cause were plotted using cumulative incidence estimates and compared using Fine and Gray competing risk regression with non-cardiac death as a competing risk. Multivariable Fine and Gray regression models were carried out separately for each cardiac dose parameter to avoid multi-collinearity. Variables were predefined for inclusion in the analysis based on known prognostic variables for survival in lung cancer (PS, stage, histology, tumour laterality and receipt of chemotherapy) and heart disease (sex, deprivation index and smoking status).Variables in which data on more than 25% of patients were missing were excluded from analysis Full death

10.3389/fonc.2022.1007577

TABLE 1 ICD10 codes to be used to identify cardiac comorbidities and death with a cardiac cause.

ICD10 Code	Meaning
	Hypertensive Heart Disease
I11	Hypertensive heart disease
I13	Hypertensive heart and renal disease
	Ischaemic Heart Disease
I20	Angina
I21	Acute myocardial infarction
I22	Subsequent myocardial infarction
I23	Complications after myocardial infarction
I24	Acute ischaemic heart disease
I25	Chronic ischaemic heart disease
	Pericardial disease
I30	Acute pericarditis
I31	Other diseases of pericardium
I32	Pericarditis in diseases classified elsewhere
	Valve disease
I33	Acute and subacute endocarditis
I34	Mitral valve disorder
I35	Aortic valve disorder
I36	Tricuspid valve disorder
I37	Pulmonary valve disorder
I38	Endocarditis, valve unspecified
I39	Endocarditis and heart valve disorder in diseases classified elsewhere
	Myocardial disease
I40	Acute myocarditis
I41	Myocarditis in diseases classified elsewhere
I42	Cardiomyopathy
I43	Cardiomyopathy in diseases classified elsewhere
	Arrhythmia
I44	Atrioventricular block and left bundle branch block
I45	Other conduction disorders
I46	Cardiac arrest
147	Paroxysmal tachycardia
I48	Atrial fibrillation
I49	Other cardiac arrhythmias
I50	Heart failure
I51	Complications and ill-defined descriptions of heart disease
I52	Other heart disorders in diseases classified elsewhere

certificate information was not available for 27 patients and these were excluded from the Fine and Gray analysis.

Results

Clinical characteristics

Linked individual patient data were available from UKCAT and NCRAS for 3100 patients treated with radiotherapy for lung cancer between 2010 and 2016. Two thousand and fourteen patients were treated with hypofractionated radiotherapy and cardiac dosimetric parameters were available for 967 patients (Figure 1).

The clinical characteristics of the 967 patients treated with hypofractionated radiotherapy and had a available cardiac dosimetric parameters are shown in Table 2. Patients with at least one cardiac comorbidity were significantly older (median age 75.8 v 72.2 years). Significantly more patients with a cardiac comorbidity were male and were ex-smokers. The cohort of patients without a cardiac comorbidity contained more patients with N3 disease. There was no difference in the dosimetric parameters or the PTV volume between the 2 groups.

Ischaemic heart disease was the most common pre-existing cardiac condition, as it was recorded in the HES of 200 (21.9%) of patients. One hundred and fifty-five patients (17.0%) were recorded as having an arrythmia, 28 patients (3.1%) were recorded as having valve disease and 9 patients (1%) with pericardiac or myocardial disease.

Death with a cardiac cause

There were 110 patients (11.4%) treated with hypofractionated radiotherapy who died with a cardiac cause. The most common cardiac event on death certificates was ischaemic heart disease (7.5%) followed by heart failure (2.6%) and cardiac arrythmia (1.6%) (Table 3).

Overall survival

Graphs of the scaled Schoenfeld residuals demonstrate that the assumption of proportional hazards is supported for the variables of interest (Supplementary Figure 1).

MHD was significantly associated with worse all cause mortality on multivariable cox analysis (adjusted HR 1.03, CI 1.01-1.03, p=0.001) as were heart V5Gy (aHR 1, CI 1.00-1.01, p=0.03), V30Gy (aHR 1.02, CI 1.01-1.03, p=0.001) and V50Gy (aHR 1.03, CI 1.01-1.05, p=0.01). Age at radiotherapy (aHR1.02, CI 1.01-1.03, p=0.001) male sex (aHR 1.21, p=0.02), T4 tumours (aHR 1.50, p=0.004), mean lung dose (aHR 1.04, p=0.002) and deprivation quintile 4 (aHR 1.40, p=0.016) were all associated

with increased hazard of death at all heart dose parameters (Supplementary Table 1). Presence of a cardiac comorbidity prior to radiotherapy was not associated with all-cause overall survival.

Death with a cardiac cause

Patients with pre-existing cardiac comorbidities

Patients with a cardiac comorbidity had an increased risk of death with a cardiac cause compared with those without a cardiac comorbidity (2-year cumulative incidence rate 21.3% v 6.2%, p<0.001, Figure 2).

The median heart V30Gy for the whole cohort was 15%. Heart V30Gy ≥ 15% was associated with a significantly higher cumulative incidence of death with a cardiac cause compared to patients with heart V30Gy <15% (2-year rate 25.8% v 17.3%, p=0.05, Figure 3A) in patients with a pre-existing cardiac comorbidity.

There was no statistically significant difference in the cumulative incidence of death with a cardiac cause in patients with a cardiac comorbidity when comparing MHD < 10Gy to MHD ≥10Gy (2-year incidence 18.1% v 23.3%, p= 0.3). Nor was there a difference in cumulative incidence of death with a cardiac cause with heart V5Gy split at a median of 48% (2-year cumulative incidence 22.8% compared to 21.4%) and heart V50Gy split at a median of 4% (2-year cumulative incidence 22.6% v 20.1%) (Figure 3).

After adjustment for tumour and cardiac risk factors using multivariable Fine and Gray analysis for cardiac death with other causes of death as a competing risk, increasing age at the start of radiotherapy (aHR 2.07, p=0.002), male sex (aHR 1.99, p=0.03) and right sided tumours (aHR 2.71, p=0.004) were associated with an increased risk of death with a cardiac cause for all heart dose parameters (Table 4). Higher cardiac V30Gy was associated with an increased risk of death with a cardiac cause (aHR 1.03, 1.00-1.07, p=0.04) but not MHD, cardiac V5Gy or cardiac V50Gy.

Patients without a pre-existing cardiac comorbidity

There was no significant difference in 2-year cumulative incidence of death with a cardiac cause in patients without a cardiac comorbidity for MHD, cardiac V30Gy, V5Gy or V50Gy. The 2-year cumulative incidence of death with a cardiac cause in patients without a cardiac comorbidity was 5.1% in patients with heart V30Gy <15% and 6.4% in patients with heart V30Gy≥ 15% (p=0.45). For patients with MHD<10Gy 2-year cumulative incidence of death with a cardiac cause was 4.5% compared to 6.9% for patients with MHD ≥10Gy (p=0.29). Cumulative incidence curves are shown in Supplementary Figure 2.

After adjusting for tumour and cardiac risk factors, increasing age at the start of radiotherapy and cardiac dosimetric parameters were associated with death with a cardiac cause (Table 5). The adjusted HR for death with a cardiac cause for MHD was 1.07 (1.01-1.13, p=0.021), for heart V5Gy 1.01 (1-1.13, p=0.05) and for heart V30Gy 1.04 (1-1.07, p=0.039).

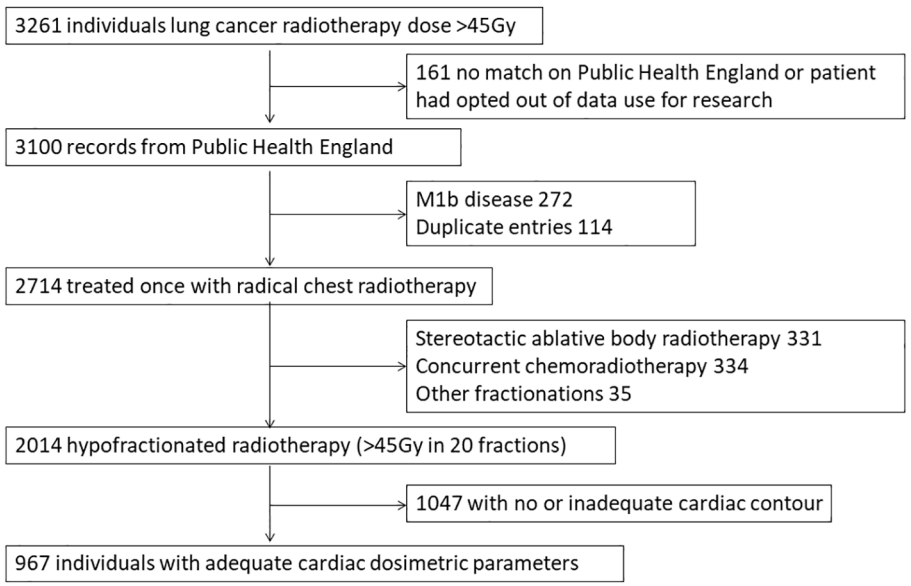

FIGURE 1
Flow diagram of patients included in final analysis

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TABLE 2 Patient and treatment characteristics.

		No cardiac comorbidity n=675	Cardiac comorbidity n=292	Total	P
Age at start of radiotherapy	Median (IQR)	72.2 (13.3)	75.8 (10.7)	73.5 (12.6)	**<0.001**
Sex	Female	340 (50.4)	112 (38.4)	452 (46.7)	**0.001**
	Male	335 (49.6)	180 (61.6)	515 (53.3)	
Performance Status	0	55 (8.1)	20 (6.8)	75 (7.8)	0.348
	1	317 (47.0)	123 (42.1)	440 (45.5)	
	2	241 (35.7)	115 (39.4)	356 (36.8)	
	3	57 (8.4)	31 (10.6)	88 (9.1)	
	(Missing)	5 (0.7)	3 (1.0)	8 (0.8)	
Histology	NSCLC	481 (71.3)	223 (76.4)	704 (72.8)	0.256
	clinical diagnosis	93 (13.8)	34 (11.6)	127 (13.1)	
	SCLC	101 (15.0)	35 (12.0)	136 (14.1)	
T stage	T1	99 (14.7)	47 (16.1)	146 (15.1)	0.366
	T2	235 (34.8)	111 (38.0)	346 (35.8)	
	T3	175 (25.9)	80 (27.4)	255 (26.4)	
	T4	155 (23.0)	53 (18.2)	208 (21.5)	
	(Missing)	11 (1.6)	1 (0.3)	12 (1.2)	
N stage	N0	227 (33.6)	107 (36.6)	334 (34.5)	**0.022**
	N1	111 (16.4)	59 (20.2)	170 (17.6)	
	N2	223 (33.0)	99 (33.9)	322 (33.3)	
	N3	111 (16.4)	27 (9.2)	138 (14.3)	
	(Missing)	3 (0.4)	0 (0.0)	3 (0.3)	
Deprivation	least deprived	92 (13.6)	37 (12.7)	129 (13.3)	0.304
	2	70 (10.4)	28 (9.6)	98 (10.1)	
	3	96 (14.2)	53 (18.2)	149 (15.4)	
	4	149 (22.1)	74 (25.3)	223 (23.1)	
	most deprived	268 (39.7)	100 (34.2)	368 (38.1)	
Smoking Status	Current	196 (29.0)	54 (18.5)	250 (25.9)	**0.004**
	Ex-smoker	312 (46.2)	161 (55.1)	473 (48.9)	
	Never	10 (1.5)	7 (2.4)	17 (1.8)	
	Not known	157 (23.3)	70 (24.0)	227 (23.5)	
Laterality	Left	291 (43.1)	113 (38.7)	404 (41.8)	0.221
	Right	383 (56.7)	179 (61.3)	562 (58.1)	
	(Missing)	1 (0.1)	0 (0.0)	1 (0.1)	
neo-adjuvant chemotherapy	No	525 (77.8)	232 (79.5)	757 (78.3)	0.621
	Yes	150 (22.2)	60 (20.5)	210 (21.7)	
MHD	Median (IQR)	12.8 (1.8-24)	12.6 (1.2-24.1)	12.8 (1.7-23.9)	0.653
Heart V5Gy	Median	48.3	47.2	47.9	0.663
Heart V30Gy	Median	15.4	13.8	14.9	0.197
Heart V50Gy	Median	4.1	4.2	4.1	0.602
MLD	Median	12.7	12.4	12.7	0.463
PTV volume	Median	324.5	306.0	318.0	0.414

NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; MHD, mean heart dose. The bold values represent variables with statistically significant p values within the table.

Discussion

In line with existing literature this study, combining population data with radiotherapy data, demonstrates how cardiac radiation dose and cardiac comorbidities both contribute to subsequent cardiac mortality in patients undergoing thoracic radiotherapy.

This is the largest published cohort investigating survival, cardiac outcomes and cardiac radiation dose in patients treated with curative hypofractionated radiotherapy for lung cancer, accounting for existing cardiac comorbidities. Unlike other cohorts it includes patients with early-stage disease and the majority of patients (78%) did not receive chemotherapy before or during radiotherapy.

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TABLE 3 Number of death certificated mentions of cardiac events.

Cardiac event	Death certificate mentions (% of total patients)
Ischaemic Heart Disease (I20-I25)	73 (7.5%)
Heart failure (I50, I51)	25 (2.6%)
Arrythmia (I44-I49)	15 (1.6%)
Pericardial and myocardial (I30-I32, I40-I43)	6 (0.6%)
Valve (I34-I37)	5 (0.5%)

This study adds to existing literature showing that increasing MHD (9, 13, 14, 18), heart V30Gy (19), heart V50Gy (9) and heart V5Gy (12) are all associated with worse overall survival in patients having curative radiotherapy for lung cancer. Moreover, we show that mean lung dose is significantly associated with survival, demonstrating that lung dose remains important in addition to heart dose.

Patients with lung cancer have poor survival due to their disease and comorbidities, therefore this study used data on cause of death from death certificates and Fine and Gray analysis to take account of the competing risk of death from other causes. Moreover, variables of interest were pre-defined prior to analysis based on previous studies to include variables that are important for outcome in lung cancer and cardiac disease to avoid over-fitting. Pre-defined variables allowed consistent analysis across different populations and dose parameters, compared to the technique of variable selection (20) which has been used in other studies (9, 13, 14).

We found that patients with known cardiac comorbidities had a 2-year cumulative incidence of cardiac-related death of 21.3% compared to 6.2% in those without a cardiac comorbidity. Similarly, in their study of 125 patients with lung cancer treated in radiotherapy dose escalation studies, Dess et al. (13) found the rate of grade ≥3 cardiac events at 2 years was 21% in patients with pre-existing cardiac disease and 7% in patients without pre-existing cardiac disease. Atkins et al. (14) describe a lower rate of major adverse cardiac events at 2 years of 11.7% in patients with heart disease and 2.5% in those without heart disease, in a cohort of 748 patients with locally advanced lung cancer treated with radiotherapy.

Both Dess and Atkins found that MHD ≥10Gy was associated with an increased rate of MACE/grade ≥3 cardiac events on univariable analysis in patients with pre-existing cardiac disease. Our study found that although patients with MHD≥10Gy had an increased incidence of cardiac death, at 23.3% compared with 18.1% if MHD<10Gy, this was not statistically significant. Heart V30Gy ≥15% in patients with

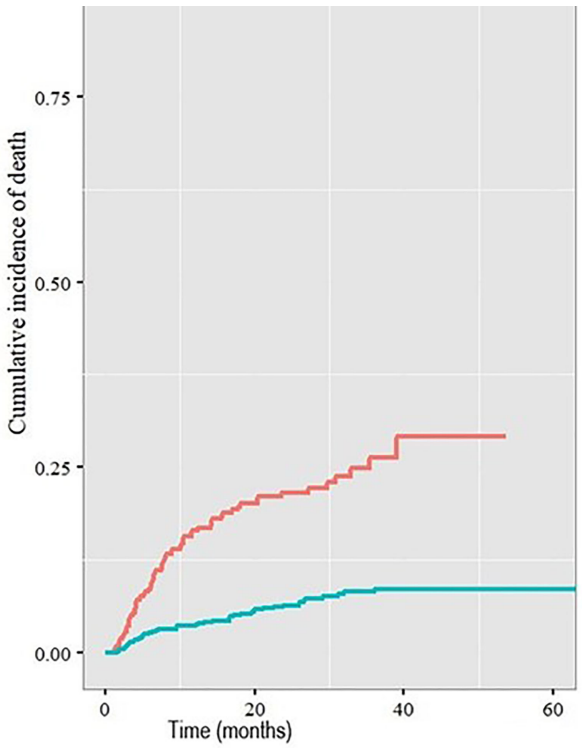

FIGURE 2
Cumulative incidence of death with a cardiac cause in patients with (red line) and without (blue line) pre-existing cardiac comorbidities.

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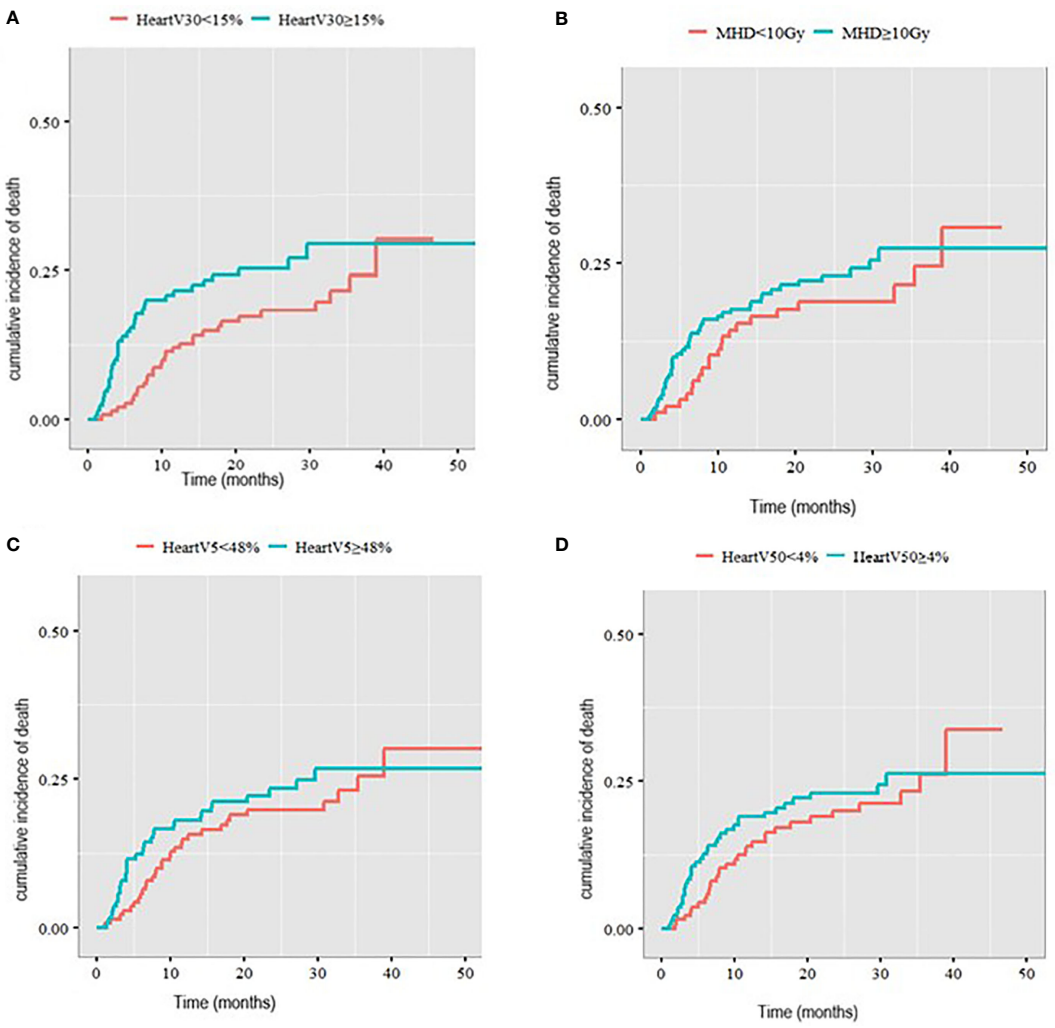

FIGURE 3
Cumulative incidence of death with a cardiac cause in patients with pre-existing cardiac comorbidities stratified by **(A)** V30Gy, **(B)** mean heart dose, **(C)** heart V5Gy, **(D)** heart V50Gy.

cardiac comorbidities was the dose parameter significantly associated with an increased incidence of cardiac death in our study, with a 2-year rate of 25.8% compared with 17.3% if heart V30Gy <15%. Once adjustment was made for other cardiac and tumour factors, the association between heart V30Gy and cardiac death remained with an adjusted HR of 1.03 (1-1.07). Increasing age and male sex were associated with cardiac death, and patient deprivation also demonstrated significance in the heart V30Gy model. Male sex, age and deprivation are all know cardiac risk factors (21) demonstrating their contribution to cardiac-related deaths following radiotherapy. The weak association between cardiac dose and death involving a cardiac cause in patients with known cardiac comorbidities may indicate that radiation exposure to the whole heart results in only a small relative increase in a cohort of patients already at high risk of cardiac events. The absolute increase in cumulative incidence of death involving a cardiac cause at 2-years comparing MHD<10Gy vs MHD>10Gy is 5% in patients with a known cardiac comorbidity and 3% in those without a cardiac comorbidity.

In patients without a known cardiac comorbidity, the 2-year cumulative incidence of cardiac death was slightly higher in patient with heart V30Gy ≥ 15% compared to V30Gy < 15% (6.4% v 5.1%) and MHD ≥10Gy compared to MHD < 10Gy (6.9% v 4.6%) however, these were not statistically significant. On adjustment for tumour and patient factors, MHD (HR 1.07, 10.1-1.13), heart V30Gy (HR 1.04, 1.0-1.07) and heart V5Gy (HR 1.01, 1.0-1.03) were associated with death with a cardiac cause. Age was also associated with cardiac-related death in those without cardiac comorbidity however, in contrast to the

TABLE 4 Fine and Gray analysis of death with a cardiac cause in patients with a pre-existing cardiac comorbidity.

Variable	aHR MHD	aHR V5Gy	aHR V30	aHR V50
Age at radiotherapy (continuous)	**1.06 (1.01-1.1, p=0.015)**	**1.05 (1.01-1.1, p=0.021)**	**1.05 (1.01-1.11, p=0.009)**	**1.06 (1.01-1.13, p=0.01)**
Sex (female v male)	**2.01 (1.08-3.75, p=0.02)**	**1.99 (1.06-3.69, p=0.03)**	**2.07 (1.105-2.39, p=0.02)**	**2.07 (1.11-3.56, p=0.02)**
Performance Status (PS 0 ref)				
PS 1	1.78 (0.46-6.83, p=0.4)	1.56 (0.43-5.64, p=0.5)	1.55 (0.42-5.74, p=0.52)	1.62(0.44-5.98, p=0.19)
PS 2	1.90 (0.52-6.81, p=0.33)	1.81 (0.54-6.1, p=0.34)	1.97 (0.56-6.94, p=0.29)	2.04 (0.58-7.18, p=0.27)
PS 3	2.96 (0.71-12.38, p=0.14)	2.91 (0.73-11.61, p=0.13)	2.88 (0.69-11.99, p=0.74)	3.4 (0.83-13.95, p=0.09)
T stage (T1 ref)				
T2	0.44 (0.19-1.02, p=0.06)	**0.45 (0.20-0.98, p=0.04)**	**0.43 (0.19-0.93, p=0.03)**	**0.43 (0.20-0.94, p=0.03)**
T3	0.5 (0.23-1.1, p=0.08)	0.47 (0.22-1.03, p=0.06)	**0.42 (0.20-0.93, p=0.03)**	**0.44 (0.2-0.95, p=0.04)**
T4	0.61 (0.25-1.5, p=0.28)	0.57 (0.23-1.41, p=0.22)	0.52 (0.22-1.2, p=0.13)	0.52 (0.22-1.22, p=0.13)
N stage (N0 ref)				
N1	0.61 (0.24-1.54, p=0.29)	0.67 (0.27-1.67, p=0.38)	0.55 (0.22-1.40, p=0.21)	0.59 (0.23-1.51, p=0.27)
N2	1.41 (0.70-2.81, p=0.75)	1.56 (0.79-3.07, p=0.2)	1.22 (0.61-2.45, p=0.58)	1.29 (0.64-2.59, p=0.48)
N3	0.82 (0.24-2.18, p=0.75)	0.95 (0.26-3.49, p=0.94)	0.74 (0.21-2.63, p=0.64)	0.77 (0.22-2.72, p=0.68)
Deprivation quintile (Q1, least deprived ref)				
2	0.74 (0.16-3.39, p=0.69)	0.78 (0.18-3.6, p=0.77)	0.84 (0.19-3.84, p=0.82)	0.85 (0.18-3.98, p=0.84)
3	1.65(0.62-4.39, p=0.31)	1.79 (0.7-4.61, p=0.23)	1.79 (0.21-2.63, p=0.64)	1.79 (0.70-4.56, p=0.22)
4	2.39 (0.97-5.9, p=0.06)	2.39 (0.97-5.92, p=0.06)	**2.53 (1.03-6.25, p=0.04)**	**2.51 (1.02-6.18, p=0.05)**
5	1.38 (0.52-3.71, p=0.52)	1.44 (0.54-3.84, p=0.47)	1.34 (0.50-3.61, p=0.56)	1.40 (0.52-3.79, p=0.51)
Laterality (left v right)	**2.72 (1.37-5.41, p=0.004)**	**2.56 (1.27-5.16, p=0.008)**	**2.71 (1.39-5.28, p=0.004)**	**2.65 (1.37-5.13, p=0.004)**
Chemotherapy prior to radiotherapy (no v yes)	0.85 (0.33-2.16, p=0.73)	0.98 (0.40-2.39, p=0.97)	0.97 (0.40-2.35, p=0.95)	1.00 (0.42-2.40, p=0.99)
Heart dose parameter	1.04 (0.99-1.09, p=0.13)	1.01 (0.99-1.02, p=0.43)	**1.03 (1.00-1.07, p=0.04)**	1.06 (0.99-1.14, p=0.10)
Mean lung dose	0.98 (0.89-1.08, p=0.69)	1.00 (0.91-1.11, p=0.96)	0.94 (0.85-1.04, p=0.21)	1.00 (0.93-1.09, p=0.91)

Number of patients analysed = 273. The bold values represent variables with statistically significant p values within the table.

group with a cardiac comorbidity, tumour laterality, deprivation and male sex were not.

The difference In variables that influence cardiac-related death between the cardiac comorbidity and no cardiac comorbidity groups would seem to suggest that heart dose is of particular importance in patients without a cardiac comorbidity. It is possible, however, that many of these patients have undiagnosed cardiac disease prior to starting treatment which could subsequently manifests after radiotherapy. In addition, patients with a previous cardiac event will receive cardiac risk factor management, as demonstrated by the significantly higher number of ex-smokers in the cardiac comorbidity group. Smoking is known to result in worse survival after radiotherapy (22). Furthermore, cardiac medications such as statins (23) and angiotensin converting enzyme inhibitors, which are prescribed routinely in patients after a cardiac event, may provide protection again radiation induced cardiac toxicity (24).

In patients with a cardiac comorbidity, right sided tumours were associated with cardiac death at all dose parameters tested, with an adjusted HR of 2.56-2.72 depending on which parameter was included in the analysis. Our group has previously shown that patients with right-sided lung tumours have worse overall survival (25) and other studies have found that dose to the right side of the heart is associated with cardiac events and death (26–28). The sino-atrial node, which generates the electrical impulse that stimulates the heart to beat, is located in the superior right atrium and the atrio-ventricular node, which co-ordinates ventricular contraction, is located in the posterior-inferior right atrium. We hypothesise that patients with an already vulnerable heart are particularly sensitive to irradiation of these essential cardiac conduction substructures.

This study only used patients who had a cardiac contour at the time of radiotherapy in order to standardise planning techniques and minimise any impact of improvements in plan optimisation when the heart was included. To overcome this issue, future work should use automatic segmentation of whole heart and substructures to examine impact of substructure dose on outcome as there is conflicting evidence and poor physiological understanding of the radiation sensitive regions of the heart.

The main limitation of this study relates to the shortcomings of using population-based data to identify cardiac comorbidities and outcomes. In our study, patients were only recorded as having a cardiac problem if they had been admitted to hospital and had a relevant entry on Hospital Episode Statistics prior to radiotherapy. Consequently, some patients with cardiac comorbidities may have been missed, although our method

TABLE 5 Fine and Gray analysis of death with a cardiac cause in patients with no cardiac comorbidity.

Variable	aHR MHD	aHR V5Gy	aHR V30Gy	aHR V50Gy
Age at radiotherapy (continuous)	**1.05 (1.02-1.09, p=0.004)**	**1.05(1.01-1.09, p=0.008)**	**1.05 (1.01-1.1, p=0.008)**	**1.05 (1.01-1.08, p=0.01)**
Sex (female v male)	1.21 (0.62-2.35, p=0.57)	1.23 (0.64-2.38, p=0.53)	1.22 (0.62-2.39, p=0.56)	1.22 (0.06-2.35, p=0.55)
Performance Status (PS 0 ref)				
PS 1	0.38 (0.09-1.53, p=0.17)	0.72 (0.09-1.50, p=0.16)	0.38 (0.09-1.64, p=0.2)	0.38 (0.09-1.61, p=0.19)
PS 2	0.80 (0.21-3.02, p=0.75)	0.81 (0.21-3.06, p=0.75)	0.80 (0.2-3.14, p=0.74)	0.81 (0.21-3.16, p=0.76)
PS 3	0.78 (0.15-3.95, p=0.76)	0.75 (0.15-3.85, p=0.73)	0.75 (0.14-3.93, p=0.74)	0.71 (0.13-3.7, p=0.76)
T stage (T1 ref)				
T2	0.91 (0.32-2.56, p=0.85)	0.52 (0.34-2.60, p=0.90)	0.96 (0.34-2.73, p=0.94)	1.05 (0.37-2.94, p=0.93)
T3	0.95 (0.31-2.95, p=0.93)	1.01 (0.33-3.10, p=0.99)	1.00 (0.33-3.07, p=0.99)	1.088 (0.36-3.2, p=0.88)
T4	1.53 (0.51-4.56, p=0.45)	1.74 (0.57-5.30, p=0.33)	1.54 (0.52-4.58, p=0.44)	1.70 (0.56-5.13, p=0.35)
N stage (N0 ref)				
N1	0.89 (0.38-2.07, p=0.78)	0.94 (0.39-2.23, p=0.89)	0.83 (0.35-1.98, p=0.67)	0.92 (0.38-2.21, p=0.85)
N2	0.67 (0.28-1.59, p=0.36)	0.77 (0.32-1.86, p=0.56)	0.58 (0.23-1.48, p=0.56)	0.73 (0.30-1.79, p=0.49)
N3	1.81 (0.64-5.1, p=0.26)	2.06 (0.70-6.02, p=0.19)	1.37 (0.47-3.93, p=0.56)	1.74 (0.61-4.93, p=0.3)
Deprivation quintile (Q1, least deprived ref)				
2	0.94 (0.22-4.07, p=0.94)	0.95 (0.22-4.09, p=0.94)	0.97 (0.23-4.17, p=0.97)	0.93 (0.21-3.98, p=0.92)
3	0.64 (0.15-2.62, p=0.53)	0.67 (0.16-2.76, p=0.57)	0.64 (0.15-2.66, p=0.54)	0.65 (0.15-2.70, p=0.55)
4	1.98 (0.63-6.21, p=0.24)	2.09 (0.66-6.67, p=0.21)	1.93 (0.63-5.89, p=0.25)	1.95 (0.62-6.08, p=0.25)
5	1.51 (0.55-2.62, p=0.53)	1.52 (0.55-4.22, p=0.42)	1.60 (0.59-4.35, p=0.36)	1.54 (0.56-4.22, p=0.4)
Laterality (left v right)	1.08 (0.58-1.99, p=0.81)	0.98 (0.53-1.79, p=0.94)	1.08 (0.58-2.00, p=0.81)	0.92 (0.49-1.72, p=0.78)
Chemotherapy prior to radiotherapy (no v yes)	0.21 (0.04-1.04, p=0.06)	0.214 (0.04-1.06, p=0.06)	0.35 (0.12-1.07, p=0.07)	0.20 (0.04-0.99, p=0.05)
Heart dose parameter	**1.07 (1.01-1.13, p=0.021)**	**1.01 (1.00-1.03, p=0.05)**	**1.04 (1.00-1.07, p=0.039)**	1.01 (0.95-1.07, p=0.78)
Mean lung dose	0.92 (0.82-1.03, p=0.13)	0.94 (0.84-1.04, p=0.23)	0.94 (0.85-1.04, p=0.21)	0.99 (0.92-1.06, p=0.7)

Number of patients analysed = 631. The bold values represent variables with statistically significant p values within the table.

will have identified those patients with the most severe cardiac disease. Nevertheless, thirty percent of patients in this cohort were identified using HES as having a cardiac comorbidity which is consistent with existing literature (3, 4, 13, 14)

The 2-year cumulative incidence of death with a cardiac cause in this study is higher than the rates of cardiac events reported in other studies. This is likely due to the different endpoint definitions used in the different studies, as well as differences in study population. We used the endpoint death with a cardiac cause, defined as any mention of a cardiac cause of death on the death certificate. This is the same method that has been used to classify deaths from sepsis (29) and by Public Health England to count deaths related to severe acute respiratory syndrome coronavirus 2 (30), however, it may overestimate the number of cardiac deaths. The alternative method of using only the primary cause of death in analysis will underestimate the contribution of cardiac events in patients with cancer (31–35) as a patient's death may have multiple contributing causes that are not adequately recorded. A consensus on cardiac event recording following cancer treatment would help to guide future work in cardio-oncology.

In this large, retrospective study of patients treated with radical, hypofractionated radiotherapy for lung cancer we show the complex interplay between patient comorbidities and heart dose in predicting future cardiac events and survival. Pre-existing heart disease is a pre-disposing factor for future cardiac death following radiotherapy, regardless of cardiac dose, therefore, cardiac surveillance should be considered before and after treatment. Further work incorporating cardiac medication data is required to understand the potential protective effects of medications in patients having thoracic radiotherapy. Secondly, stricter heart dose parameters should be used in thoracic radiotherapy. The quantitative analysis of normal tissue effects in the clinic (QUANTEC) suggests V30Gy <46% however, our study shows that V30Gy <15% may be a more appropriate threshold, especially in patients with pre-existing cardiac disease. Achieving stricter heart dose parameters may be possible with advanced technologies such as proton therapy. Further understanding of the radiation sensitivity of cardiac substructures, such as the base of the heart containing the conduction system, in patients with and without cardiac risk factors will enable better cardiac sparing in the future.

Data availability statement

KB, CF-F and AMcW conceived the study. FS, AMcW and KF obtained data and ethical approvals from Public Health England. KB and AA analysed the data. KB wrote the

manuscript with assistance from all authors. All authors contributed to the article and approved the submitted version.

Ethics statement

The studies involving human participants were reviewed and approved by Leeds East Research Ethics Committee (18/YH/0058). The ethics committee waived the requirement of written informed consent for participation.

Author contributions

KB, CF-F and AMcW conceived the study. FS, AMcW and KF obtained data and ethical approvals from Public Health England. KB and AA analysed the data. KB wrote the manuscript with assistance from all authors. All authors contributed to the article and approved the submitted version.

Funding

This work is supported by Yorkshire Cancer Research [M401] and by Cancer Research UK via funding to the Cancer Research UK Manchester Centre [C147/A25254] and their Clinical Academic Training Award [C19941/A28707]. CF-F and MvH are supported by NIHR Manchester Biomaedical Research Centre.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.1007577/full#supplementary-material

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