Clinical and Translational Oncology
https://doi.org/10.1007/s12094-022-03024-1

RESEARCH ARTICLE

Radiation pneumonitis in relation to pulmonary function, dosimetric factors, TGFβ1 expression, and quality of life in breast cancer patients receiving post-operative radiotherapy: a prospective 6-month follow-up study

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Received: 9 August 2022 / Accepted: 21 November 2022

Abstract
Purpose To investigate development of radiation pneumonitis (RP) in relation to pulmonary function, dosimetric factors, and transforming growth factor beta-1 (TGFβ1) expression in irradiated breast cancer patients.
Methods A total of 49 breast cancer patients who received post-operative radiotherapy (RT) were evaluated in terms of pulmonary function tests (PFTs), quality of life (QoL), development of RP, dosimetric factors, cytokine levels, and lung high-resolution computed tomography (HRCT) before and after RT. ROC analysis was performed for performance of dosimetric factors in predicting RP, while frequencies of single nucleotide polymorphisms (SNPs) genotyped for TGFβ1 (rs11466345 and rs1800470) were also evaluated.
Results All cases with RP (10.2%) recovered clinically at the end of third post-RT month. PFT and HRCT parameters were similar before and after RT overall, as well as by RP and the radiation field subgroups. ROC analysis revealed the significant role of the ipsilateral V5 (cutoff value of 45.9%, $p=0.039$), V10 (29.4%, $p=0.015$), V20 (23%, $p=0.017$), and MLD (1200 cGy, $p=0.030$) in predicting RP. Higher post-RT TGFβ1 levels ($p=0.037$) were noted overall and in patients with RP. Patient and control groups were similar in terms of frequencies of SNPs genotyped for TGFβ1 (rs11466345 and rs1800470). EORTC QLQ-C30 and QLQ-BR-23 scores were similar in patients with vs. without RP.
Conclusion Our findings revealed significant role of dosimetric factors including MLD, V20 as well as the low dose-volume metrics in predicting the risk of RP among breast cancer patients who received post-operative RT. Implementation of RT, extent of radiation field or the presence of RP had no significant impact on PFTs.

Keywords Breast cancer · Radiotherapy · Radiation pneumonitis · Pulmonary function · Dosimetric factors · TGFß1

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Published online: 08 December 2022

Introduction

Breast cancer patients are the third most commonly affected population by radiation-induced lung injury (RILI) which manifests acutely as radiation pneumonitis (RP) after the lung cancer and mediastinal lymphoma patients [1–3]. Also, a growing number of cancer survivors are potentially at risk for the late effects of radiation which manifests chronically as radiation-induced pulmonary fibrosis (RIF) [2–4]. Accordingly, preventing and reducing the radiation-induced toxicities of normal tissues, as well as identifying those who are at an increased risk of RILI have become increasingly important components in the treatment plan to enable a safer and more beneficial RT [4–6].

The involvement of cytokine-mediated signal cascades (i.e., transforming growth factor beta-1 [TGF-β1]) has been suggested in the pathogenesis of radiation pneumonitis RP [2, 4], while specific single nucleotide polymorphisms (SNPs) in candidate genes have been associated primarily with radiation-related processes (i.e., TGFβ1) [4, 6]. However, the significance of all these risk factors remains unclear due to the rarity of RP [1], while the exact relationship between PFT parameters or dosimetric parameters for the ipsilateral lung irradiation and RP has also not yet been fully established [4, 7].

This study aimed to investigate the incidence of RP in relation to pulmonary function, dosimetric factors, TGFβ1 expression, and QoL in breast cancer patients receiving postoperative RT through a prospective 6-month follow-up.

Materials and methods

Study population

A total of 49 patients with breast cancer who received postoperative RT were included in this prospective 6-month follow-up study conducted at a tertiary care radiation oncology clinic between 2014 and 2016. Breast cancer patients (aged 18–75 years) operated with modified radical mastectomy or lumpectomy and planned to receive conformal or intensity modulated RT (IMRT) were followed up to 6 months.

Written informed consent was obtained from each subject and the study was conducted in accordance with the ethical principles stated in the "Declaration of Helsinki" and approved by the institutional ethics committee.

Assessments

Data on patient age, type of surgery, menopausal status, smoking status, comorbidities, and treatment characteristics (type of chemotherapy, radiation field, and dosimetry) were recorded at baseline prior to RT. Pulmonary function tests (PFTs) including forced vital capacity (FVC), forced expiratory volume at the 1st second (FEV1), FEV1/FVC ratio, peak expiratory flow rate (PEF), and forced expiratory flow from 25 to 75% (FEF 25–75%) were recorded both before and after (third month and sixth month) the RT. The QoL assessment with European Organization for Research and Treatment of Cancer (EORTC) QoL questionnaires (QLQ-C30 and QLQ-BR23) were performed before the RT, after the last dose of RT, and 3 months after the RT. Development of RP, dosimetric factors including the volume of the ipsilateral lung receiving 5 Gy (V5), 10 Gy (V10), 20 Gy (V20), and 30 Gy (V30) and the mean lung dose (MLD), as well as the TGFß1, IL-6, and IL-8 plasma levels and lung HRCT imaging findings were also recorded before and after RT.

ROC analysis was performed for performance of dosimetric factors in predicting RP, while frequencies of SNPs genotyped for TGFβ1 (rs11466345 and rs1800470) were also evaluated in control and patient groups.

RT techniques

Treatment planning was done at three-dimensional planning system (Eclipse Planning System, v11.0). Conformal planning (field in field FinF) or reverse planning IMRT techniques were used for all patients. Patients received 50 Gy (range, 46–50 Gy) radiation to whole breast or chest wall median and then a total of 60 Gy to the tumor site (boost dose 10 Gy). In locally advanced stages, supraclavicular and axilla fields were included. Ipsilateral internal mammary field was also involved in two patients. Treatment was applied in linear accelerator (Trilogy, Varian) and completed in 6 weeks (range, 5–7 weeks) in all patients. Dosimetric results were revealed from dose-volume histograms. The percentage of mean volume of lung receiving radiation doses of 5 Gy (V5), 10 Gy (V10), 20 Gy (V20) and 30 Gy (V30) and the MLD were calculated. Overall, neoadjuvant chemotherapy, adjuvant chemotherapy, and hormone therapy alone were applied in 3, 38, and 8 patients, respectively.

Cytokine levels and polymorphism analysis

Blood samples were collected to determine the plasma cytokine levels (TGF-β1, IL-6, IL-8) and SNPs genotyped for TGFβ1 (rs1800470 and rs11466345 polymorphisms). TGFβ1, IL-6, and IL-8 levels in blood serum were quantitatively detected by enzyme-linked immunosorbent assays (ELISA) using commercial kits, according to the protocols recommended by the manufacturers (Boster Biological Technology, CA, USA). Frequencies of SNPs were determined using DNA isolation kit and region-specific probes (High Pure PCR Template Preparation Kit, Roche

Applied Science) via real-time polymerase chain reaction (RT-PCR) (LightCycler 96, Roche). After DNA isolation, all samples were scanned with TaqMan SNP Genotyping Assay kit synthesized for two different genotypes, rs1800470 and rs11466345. rs1800470 SNP region is located on Chr.19: 41353016, while rs11466345 SNP region is located on Chr. 19:41337556 and the probe sequences were "gctgcggctgctgc[C/G/T] gctgctgctaccgctgctgtggcta" for rs1800470 and "caggtatggtagctcac[A/G]cccgtaatcccagcactttgggagg" for rs11466345. The results were evaluated using GraphPad Prism, v.5.0.

Assessments for RP and PFTs

Clinical examination for lung injury and PFTs were performed by two pulmonologists (EE, SK) before and after (third and sixth months) the RT. RP was diagnosed based on clinical signs with exclusion of infection or any other reason for pneumonia [8]. Toxicity was graded due to the modified RTOG/SWOG/CTC criteria including Grade 0 (no change of respiratory symptoms compared with pre-treatment evaluation), Grade 1 (mild dry cough or dyspnea on exertion),

Grade 2 (persistent cough requiring narcotic antitussive agents or dyspnea with minimal effort but not at rest), Grade 3 (severe cough unresponsive to narcotic antitussive agents or dyspnea at rest or requirement of intermittent oxygen or steroids), and Grade 4 (severe respiratory insufficiency or need for continuous oxygen or assisted ventilation) [9].

Radiological assessment

High-resolution computed tomography (HRCT) imaging analysis of the lungs was performed on the planning CT images of RT by an experienced radiologists (CC) blinded to study protocol and evaluated based on radiological assessment criteria and categorized as 0 (no abnormality), 1 (thickened septal lines), 2 (ground-glass opacity), 3 (subpleural line), 4 (reticular and linear opacification), and 5 (consolidation) [10].

EORTC QLQ-C30 and QLQ-BR23

EORTC QLQ-C30 is a 30-item cancer-specific measure of HRQOL that consists of 9 (5 functional scales, 3 symptom scales and a global health status/QoL scale) multi-item scales, while EORTC QLQ-BR23 is a breast-specific module that comprises of 23 questions to assess body image, sexual functioning, sexual enjoyment, future perspective, systemic therapy side effects, breast symptoms, arm symptoms, and upset by hair loss [11, 12]. The reliability and validity analysis of Turkish versions of EORTC QLQ-C30 and EORTC QLQ-BR23 were performed by Cankurtaran et al. in 2008 [13] and by Demirci et al. in 2011[14], respectively. The scale scores were linearly transformed (0–100) with higher scores on the functional scales, global health status or QoL representing a high level of functioning or QoL, while higher scores on the symptom scales implying a stronger symptom burden [11, 12].

Statistical analysis

Statistical analysis was made using MedCalc Statistical Software version 12.7.7 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2013). Chi-squared test was used for the comparison of categorical data, while numerical data were analyzed using repeated measures ANOVA for variables with normal distribution and with Mann–Whitney *U* and Friedman tests for non-normally distributed variables and via Skillings–Mack test for non-normally distributed variables with missing values. Change over time is evaluated by Wilcoxon signed rank test. Receiver operating characteristic (ROC) curve was plotted to determine performance of dosimetric factors in predicting RP with calculation of area under curve (AUC) values and optimal cutoff value maximizing the Youden's index providing the best tradeoff between sensitivity and specificity. The AUCs were compared using ROC test function based on Delong's method for covariance estimation. Data were expressed as mean ± standard deviation (SD), median (minimum–maximum), 95% confidence interval (CI), and percent (%) where appropriate. $p < 0.05$ was considered statistically significant.

Results

Patient and treatment characteristics

Median patient age was 46 years (23–67 years) and 55.1% of patients were in the premenopausal status. Previous surgery was lumpectomy in 69.4% of patients, and 83.7% of patient received chemotherapy (4AC + Paclitaxel in 38.8%). RT field was breast alone in 44.9% of patients, while concomitant regional node irradiation or chest wall + regional node irradiation was applied in 24.5% and 30.6% of patients, respectively (Table 1).

All patients completed RT as planned. The RP (based on clinical signs) was observed in five (10.2%) patients after RT. Of these five patients, four patients experienced grade 2 RP, and one patients had grade 3 RP that required steroid treatment. RP recovered clinically at the end of third post-treatment month in all cases.

Pre-treatment vs. post-treatment PFTs

PFT parameters before and after RT were similar in the overall study population and by RP or radiation field (Table 2).

Table 1 Patient and treatment characteristics ($n = 49$)

Age (year), median (min–max)	46 (23–67)
Menopausal status, *n* (%)	
Premenopausal	27 (55.1)
Postmenopausal	22 (44.9)
Active smoking, *n* (%)	3 (6.1)
Comorbidities, *n* (%)	
Pulmonary disease (i.e., asthma)	5 (10.2)
Diabetes mellitus	2 (4.1)
Hypertension	3 (6.1)
Side of disease, *n* (%)	
Right	25 (51.0)
Left	24 (49.0)
Surgery, *n* (%)	
Lumpectomy	34 (69.4)
Mastectomy	15 (30.6)
Chemotherapy, *n* (%)	41 (83.7)
4AC + Paclitaxel	19 (38.8)
4AC	8 (16.3)
4AC + Paclitaxel + Herceptin	14 (28.6)
Radiotherapy field, *n* (%)	
Breast + boost	22 (44.9)
Breast + boost + axillary + subclavian (breast-RNI)	12 (24.5)
Chest wall and axillary and subclavian lymph nodes (ipsilateral internal mammary; breast/CW + RNI)	15 (30.6)

Pulmonary HRCT findings

Radiological pulmonary findings differed after RT in all patients and extensively developed in the ipsilateral RT tangential fields. Patients with and without RP had similar HRCT findings, including septal thickening and subpleural opacities in most of cases.

Figure 1 illustrates the pulmonary HRCT radiologic findings in the overall study population and in patients with RP.

Performance of dosimetric factors in predicting RP

ROC analysis revealed the significant role of the ipsilateral V5 (cutoff value of 45.9%, AUC (95% CI) 0.75 (0.55–0.94), sensitivity: 60%, specificity: 86%, $p = 0.039$), V10 (cutoff value of 29.4%, AUC (95% CI) 0.80 (0.63–0.97), sensitivity: 80%, specificity: 75%, $p = 0.015$), V20 (cutoff value of 23%, AUC (95% CI) 0.79 (0.62–096), sensitivity: 80%, specificity: 79%, $p = 0.017$), and MLD (cutoff value of 1200 cGy, AUC (95% CI) 0.77 (0.58–0.95), sensitivity: 80%, specificity: 68%, $p = 0.030$) in predicting development of RP. No significant difference was noted between AUC values of dosimetric factors (Table 3).

Dosimetry findings in patients with RP are summarized in Table 4. The L-CW + RNI (chest wall + regional nodal irradiation) applied in two patients was associated with higher lung volumes for V20 (33% and 24.6%, respectively), V10 (80% and 62.6%), and V5 (98.10% and 98%) and higher MLD (1810.90 cGy and 1589.40 cGy, respectively) than the target volumes (L-CW + RNI, L-Breast and L-Breast + RNI) applied for other ($n = 3$) patients (Table 4).

Cytokine evaluations before and after RT

Mean (%95 CI) TGFβ1 level showed significant increase after RT when compared to pre-treatment levels [103.1(45.37–160.9) vs. 88.7(21.51–155.8), $p = 0.037$]. The pre-RT and post-RT values for IL-6 and IL-8 were similar. Although could not be further analyzed due to small sample size, there was a tendency for higher post-treatment TGFβ1 level also in patients with RP (Table 4).

Frequency of SNPs genotyped for TGFβ1

Due to overlap of studied regions with other SNPs, clear peak could not be obtained in samples. Based on accountable data, no significant difference was noted between patient and control groups in terms of frequencies of SNPs genotyped for TGFβ1 (rs11466345 and rs1800470). Genotype frequencies in patient and control groups were also consistent with Hardy–Weinberg equilibrium (Table 5).

EORTC QLQ-C30 and QLQ-BR-23 scores

EORTC QLQ-C30 assessment revealed improved global health status scores ($p < 0.001$) and symptom scale scores including fatigue ($p = 0.009$), pain ($p = 0.020$), and nausea/vomiting ($p = 0.032$) in the post-treatment period. No significant difference was noted before vs. after RT in functional scales and single items (Supplementary Table 1).

EORTC QLQ-BR-23 scores revealed temporary deterioration in breast symptoms and upset by hair loss after the last dose of RT, which were significantly improved at the third month of post-RT period ($p < 0.001$ for each) (Supplementary Table 1).

EORTC QLQ-C30 and QLQ-BR-23 scores were similar in patients with vs. without RP.

Discussion

In this study, 10% of breast cancer patients who received post-operative RT experienced RP which was grade 2 in most cases and recovered clinically at the end of third post-RT month in all cases. Post-RT findings on lung HRCT were similar in patients with and without RP, which included septal thickening and subpleural opacities in most of cases.

No significant change was noted in PFTs before and after RT in our cohort, regardless of development of RP,

Table 2 Pre- vs. post-treatment PFTs overall and by radiation pneumonitis and radiation field

		Pre-RT ($n=49$)	Post-RT third month ($n=46$)	Post-RT sixth month ($n=36$)	p value[1]
PFTs overall					
FVC (L)		3.28 ± 0.75	3.19 ± 0.69	3.11 ± 0.61	0.618
FEV1 (L)		2.67 ± 0.64	2.55 ± 0.54	2.56 ± 0.53	0.946
FEV1/FVC		80.97 ± 6.99	80.27 ± 8.68	82.74 ± 7.14	0.096[2]
MFEF 25–75 (%)		2.7 ± 1.05	2.66 ± 0.93	2.81 ± 0.98	0.097
PFTs in patients with RP ($n=5$)					
FVC (L)		3.65 ± 0.99	3.31 ± 0.71	3.69 ± 0.78	0.223
FEV1 (L)		2.9 ± 0.79	2.69 ± 0.59	2.86 ± 0.93	0.223
FEV1/FVC		79.63 ± 7	81.16 ± 2.93	76.5 ± 9.25	0.223
MFEF 25–75 (%)		2.65 ± 1.05	2.76 ± 0.72	2.61 ± 1.67	0.135
PFTs by radiation field					
FVC (L)	Breast	3.17 (2.1–5.58)	2.86 (1.92–4.64)	2.76 (1.89–4.29)	0.327
	Breast/CW + RNI	3.41 (2.17–4.7)	3.34 (2.44–4.89)	3.27 (2.37–4.24)	0.350
	p value[3]	0.269	**0.043**	**0.033**	
FEV1 (L)	Breast	2.54 (1.3–4.61)	2.37 (1.14–3.29)	2.24 (1.8–3.4)	0.717
	Breast/CW + RNI	2.76 (1.73–3.88)	2.65 (1.88–3.88)	2.72 (1.82–3.52)	0.784
	p value[3]	0.568	0.144	0.087	
FEV1/FVC (%)	Breast	82.64 (60.86–91.2)	84.03 (51.25–93.36)	83.3 (71.46–96.05)	0.717
	Breast/CW + RNI	81.15 (67.68–89.39)	79.38 (68.77–97.05)	82.25 (65.12–96.58)	0.241
	p value[3]	0.473	0.105	0.434	
MFEF 25–75 (%)	Breast	2.78 (0.58–4.91)	2.86 (0.54–4.45)	2.61 (1.4–4.26)	0.684
	Breast/CW + RNI	2.73 (0.83–4.3)	2.61 (1.39–4.29)	3.06 (1.4–4.88)	0.118
	p value[3]	0.540	0.458	0.870	

Values in bold indicate statistical significance ($p < 0.05$)

RT radiotherapy, *RP* radiation pneumonitis, *PFT* pulmonary function test, *FVC* forced vital capacity, *FEV1* forced expiratory volume in 1 s, *MEF25-75%* mean forced expiratory flow between 25 and 75% of FVC, *CW + RNI* chest wall + regional nodal irradiation

[1]Friedman test

[2]repeated measures ANOVA

[3]Mann-Whitney *U* test

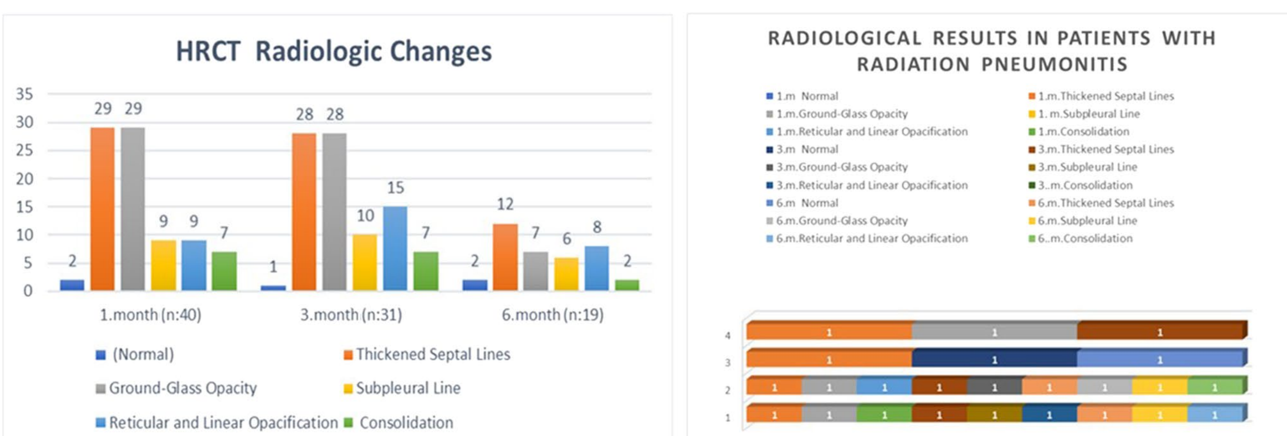

Fig. 1 Pulmonary HRCT findings before and after radiotherapy

Table 3 ROC analysis for the performance of dosimetric factors in predicting radiation pneumonitis

	Dosimetry mean ± SD	Cutoff value	AUC (%95 CI LB-UB)	*p* value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
V5 (%)	40.2 ± 14.0	45.9%	0.75 (0.55–0.94)	**0.039**	0.60	0.86	0.33	0.95
V10 (%)	27.7 ± 10.7	29.4%	0.80(0.63–0.97)	**0.015**	0.80	0.75	0.27	0.97
V20 (%)	20.0 ± 5.1	23%	0.79(0.62–096)	**0.017**	0.80	0.79	0.31	0.97
V30 (%)	16.9 ± 4.5	19.8%	0.50(0.23–0.77)	0.51	0	0.75	0.0	0.87
MLD (cGy)	1107.9 ± 244.6	1200 cGy	0.77(0.58–0.95)	**0.030**	0.80	0.68	0.22	0.97

Values in bold indicate statistical significance ($p < 0.05$)

MLD mean lung dose, *AUC* area under curve, *CI* confidence interval, *LB* lower bound, *UB* upper bound, *PPV* positive predictive value, *NPV* negative predictive value

$p > 0.05$ between AUC values of dosimetric factors

Table 4 Dosimetry findings and TGFβ1, IL-6, and IL-8 levels before and after radiotherapy

Dosimetry findings in patients with radiation pneumonitis							
Patients	Target volume	Dose	V30 (%)	V20 (%)	V10 (%)	V5 (%)	MLD (cGy)
1	L-CW + RNI	50 Gy	18.70	25.80	30.50	38.50	1218.00
2	L-Breast	60 Gy	15.80	18.10	23.10	32.30	962.30
3	L-Breast + RNI	60 Gy/50 Gy	19.60	23.60	32.50	46.20	1259.70
4	L-CW + RNI	50 Gy	14.60	33.00	80.00	98.10	1810.90
5	L-CW + RNI	50 Gy	14.40	24.60	62.60	98.00	1589.40

Cytokine levels in all patients					
	Pre-RT		Post-RT		*p* value
	Median	Mean (%95 CI)	Median	Mean (%95 CI)	
TGFβ1	34.09	88.7 (21.51–155.8)	50.98	103.1(45.37–160.9)	**0.037**
IL-6	4.16	6.9 (4.14–9.61)	4.15	18.6 (4.38–32.8)	0.459
IL-8	11.5	22.8 (14.17–31.4)	2.40	22.4 (12.13–32.7)	0.606

TGFβ1 level in patients with radiation pneumonitis			
	Pre-RT	Post-RT	
Case 1	4.34	41.4	N/A
Case 2	72.6	100	
Case 3	0	48.57	
Case 4	0	120.38	
Case 5	0	89.12	

Values in bold indicate statistical significance ($p < 0.05$)

MLD mean lung dose, *CW + RNI* chest wall + regional nodal irradiation, *RT* radiotherapy, *N/A* could not be performed due to insufficient sample size

Wilcoxon signed rank test

while HRCT imaging also revealed similar post-RT findings of lung in patients with or without RP. These findings support that RP after conformal RT in breast cancer patients associates mostly with asymptomatic minimal pulmonary radiologic change and is relieved and easily managed by supportive care with no respiratory problems remained after the first year of RT [5, 15]. However, some studies reported significant changes in PFTs and radiological findings after delivery of locoregional irradiation in breast cancer patients, including a high incidence of RP and RP-related lung HRCT findings as well as deterioration of the lung function indices (i.e., FEV1) with significant losses in respiratory and exercise capacity (particularly in those with RP) [2, 3, 16–18]. Hence, spirometry is considered helpful in identifying breast cancer patients with risk of RP given the lack of obvious clinical symptoms in patients presented with RP [2, 3, 16–18].

Table 5 Frequencies of SNPs genotyped for TGFβ1 in control and patient groups

Single nucleotide polymorphisms (SNPs) genotyped for TGFβ1											
	rs11466345						rs1800470				
	Control		Patient		p value[1]		Control		Patient		p value[1]
	n	Frequency distribution	n	Frequency distribution			n	Frequency distribution	n	Frequency distribution	
AA	40	0.85	36	0.76	0.5771	**CC**	1	0.87	2	0.76	0.4064
AG	6	0.12	10	0.21		**CT**	5	0.10	9	0.19	
GG	1	0.02	1	0.02		**TT**	41	0.02	36	0.04	
HWE test	**Control**		**Patient**			**HWE test**	**Control**		**Patient**		
$\chi 2$	0.3		0.82			$\chi 2$	2.44		1.81		
p	0.58		0.36			p	0.11		0.17		

HWE Hardy–Weinberg equilibrium

[1]Chi-squared test

The extent of radiation field had no clinically significant impact on PFTs in our study, while some studies reported that irradiation of the axillary and the supraclavicular lymph node regions (local–regional vs. simple local irradiation) was associated with a 2.5-times higher risk of RP and a twofold risk of RIF [2, 5, 7, 19]. In fact, some studies in breast cancer patients also reported that irradiation field size had no significant effect on RP incidence [1, 20, 21], while due to inconsistent findings on the relation of PFTs to RILI in the setting of lung cancer, it also remains controversial whether the impaired baseline PFT is associated with higher or lower risk of RILI [4].

Accordingly, while significant post-RT radiogenic lung sequelae are rare in breast cancer and lung density is considered to reach a stable level by 1 year in the majority of patients, implementing a longer follow-up to assess the ultimate level of late lung damage is a widely accepted practice, due to likelihood of RIF to develop after 12 months as well as the lung changes to progress for even 5–6 years after RT [3–5, 7, 17].

In the current study, the dosimetric factors including ipsilateral V5 (for a cutoff value of 45.9%), V10 (for a cutoff value of 29.4%), V20 (for a cutoff value of 23%), and MLD (for a cutoff value of 1200 cGy) were found to reveal similar AUC values and thus to equally predict the development of RP. These findings support the idea that radiation lung dosimetry is the most important treatment factor associated with the risk of RP, and emphasize the value of dosimetric predictors in assessing the RP risk of the ipsilateral lung and the likelihood of related dose-volume lung constraints to significantly reduce RP risk in breast cancer [1, 3–5, 7, 15, 21–24]. Most of the previous studies in irradiated breast cancer patients reported ipsilateral V20 [5, 7, 22] and V30 [7, 22, 25] as the most significant dosimetric factor associated with RP development. Notably, V20 (for a cutoff value of 39.8%) and V30 (for a cutoff value of 25.7%) were reported to predict the risk of RP for local–regional irradiation, while V20 (for a cutoff value of 20.2%) was shown to predict the risk of RP for breast irradiation alone [7].

Our findings indicate the risk of RP to significantly correlate even with low dose-volume metrics (i.e., V5 and V10). Likewise, in a 6-month follow-up study of 32 irradiated breast cancer patients, RP was reported to occur in 8 (25.0%) patients and V10 was reported to be the volume parameter that was significantly associated with RP incidence (5.3% for V10 < 40% and 61.5 for V10 ≥ 40%) [3]. In a multivariate analysis of determinants of pneumonitis risk after definitive RT for lung cancer, the lung V5 was reported to remain a significant predictor of grade ≥ 2 pneumonitis, even when controlling for MLD, while for MLD and V20 were statistically significant predictors of grade ≥ 3 pneumonitis [26].

Notably, given the possibility of larger lung volumes treated with lower doses to be more critical in predicting RP than smaller volumes treated with higher doses, radiation is recommended to be carefully planned when using IMRT (delivering low radiation doses to large lung volumes) [25]. In addition, for the same MLD, implementing high doses to small lung volumes ("a lot to a little") was reported to be worse than implementing low doses to large volumes ("a little to a lot") [27].

In the present study, TGFβ1 levels were significantly increased after RT and similarly in patients with and without RP, while no significant difference was noted between patient and control groups in terms of frequencies of SNPs genotyped for TGFβ1 (rs11466345 and rs1800470). Hence, our findings indicate radiation-induced elevation in plasma TGF-β1 level, regardless of the development of RP, emphasizing that TGFβ1 may not be a predictor of the risk of RP among the breast cancer patients. Likewise, some studies in breast cancer patients have reported elevated levels of TGFβ1 at the third month post-RT in patients with symptomatic RP, but no significant role noted in predicting the risk of early or late radiogenic lung changes [5], and TGFβ1 concentrations were also not considered to predict RP except when large lung volumes were irradiated [28].

In breast cancer patients, few number of studies available have revealed inconsistent findings related to association of TGFβ1gene polymorphisms with RP or RIF in breast [6, 29, 30]. Two studies demonstrated that the polymorphisms in the TGFß1 gene (SNP rs1800469 and rs1800470) were strongly associated with normal tissue toxicity and RIF in breast in patients with breast cancer after RT [29, 30], one study indicated the association of SNP rs1800469 in the TGFβ1 promoter with wound repair and late radiation toxicity in African Americans but not in whites [8] and another study identified associations of SNPs in TGFβ1 (rs4803455) with RT-induced early adverse skin reactions [31]. However, a meta-analysis with 2,782 breast cancer patients from 11 different cohorts did not confirm previous reports of an association between fibrosis or overall toxicity and rs1800469 genotype in breast cancer patients [32]. Hence, given the possibility of variance in genetic risk factors and pathogenesis of RP among different ethnic groups, a need for further large-scale studies that would account for the possibility of such ethnic heterogeneity in the radiation-induced toxicities has been emphasized to justify the use of polymorphisms as predictive markers to individualize radiation therapy on a genetic basis [6, 33].

RT-induced injury has also been associated with a systemic impairment that leads to diminished exercise capacity and worsened QoL [2, 34]. Nonetheless, in breast cancer patients receiving post-surgery local–regional RT, minimizing the dose to the ipsilateral lung to V20 < 30% was reported to relieve most of the side effects from RT and to significantly improve QoL in terms of role functioning, social functioning, and future perspective 4 months after RT compared to baseline [33]. The authors also noted that physical functioning was not affected by RT along with no changes for pain and dyspnea after RT in their series [33]. Also, in a study comparing the whole breast RT (WBRT) vs. accelerated partial breast irradiation (APBI) in terms of radiation side effects severity and quality of life in 285 newly diagnosed early stage breast cancer patients, while patients receiving WBRT (vs. APBI) reported greater increase in fatigue and skin irritation severity from 6-week to 6-month, WBRT and APBI revealed similar QoL change over 2-year follow-up [35].

Notably, our cohort patients with and without RP had similar QoL outcome. EORTC QLQ-C30 assessment revealed improved global health status scores after the RT and during 3-month post-treatment follow-up as well as improved symptom scale scores (fatigue, pain, and nausea/vomiting), while EORTC QLQ-BR-23 scores revealed temporary deterioration in breast symptoms and upset by hair loss after the last dose of RT, which were significantly improved at the third month of post-RT period.

Certain limitations of this study should be considered. First, due to single center study design with relatively small sample size, potential lack of generalizability is an important limitation, which also prevented the conduction of certain subgroup analysis regarding the presence vs. absence of RP. Second, data on HRCT of the lung were not available in one case who had been diagnosed with RP outside the study center, while spirometry (available for 46 and 36 patients at third and sixth months after RT) and thoracic HRCT imaging (available for 31 and 19 patients, respectively) could not be performed in all patients during the post-RT follow-up.

Conclusion

In conclusion, our findings revealed significant role of dosimetric factors including MLD, V20 as well as the low dose-volume metrics (i.e., V5 or V10) in predicting the risk of RP among breast cancer patients who received post-operative RT. Implementation of RT, extent of radiation field or the presence of RP had no significant impact on PFTs, whereas RT was linked to significantly higher TGFβ1 levels and better QoL outcome, regardless of RP, with no significant differences in the frequencies of SNPs (rs11466345 and rs1800470) genotyped for TGFβ1. Further studies in larger samples are needed to address the pre-RT clinical and dosimetric factors in combination with genetic characteristics for the risk of radiogenic lung sequelae after RT to develop a reliable predictive model of the risk of RILI and, thus, to improve the appropriate delivery of adjuvant RT in breast cancer patients.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12094-022-03024-1.

Funding This study was supported by Marmara University Scientific Research Projects Committee (Project Number: SAG-B-050614-0218).

Data availability Research data are stored in an institutional repository and will be shared upon request to the corresponding author.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was conducted in accordance with the ethical principles stated in the "Declaration of Helsinki" and approved by the Marmara University Faculty of Medicine Ethics Committee (Date of Approval: 28/06/2013, Reference number/Protocol No: 2013-0212).

Informed consent Written informed consent was obtained from each subject following a detailed explanation of the objectives and protocol of the study.

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