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Scientific Article

Longitudinal Analyses and Predictive Factors of Radiation-Induced Lymphopenia After Postmastectomy Hypofractionated Radiation Therapy for Breast Cancer: A Pooled Cohort Study of 2 Prospective Trials

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Purpose: Radiation-induced lymphopenia (RIL) correlates with poor prognoses in solid tumors. This study aimed to investigate the post−radiation therapy (RT) longitudinal lymphocyte changes and the impact of different RT techniques on RIL in breast cancer patients.

Methods and Materials: We prospectively assessed 607 breast cancer patients who received hypofractionated postmastectomy RT in 8 hospitals. Radiation therapy techniques included integrated photon-based intensity modulated technique (integrated RT) and a combination of photon irradiation of supraclavicular nodes and electron irradiation of the chest wall and/or the internal mammary node (hybrid RT). Peripheral lymphocyte counts (PLC) were determined before RT, weekly during RT, at 1 and 2 weeks, 3 and 6 months post−RT, and then every 6 months. The primary outcome was the nadir PLC during RT, for which associated factors were analyzed. Univariate, multivariable linear regression and propensity score matching analyses were performed to evaluate the effect of different RT techniques on nadir PLC.

Results: During RT, 121 (19.9%) patients had grade ≥3 RIL with a nadir PLC of $0.75 \pm 0.33 \times 10^9$/L. The PLC started to recover at 1 week and reached pre−RT levels 1 year after RT and higher than pre−RT levels 2 years later. Multivariate analysis identified young age, low body mass index, radiation therapy targets involving multiple regions, integrated RT, and low pre−radiation therapy PLC as independent risk factors for nadir PLC ($P < .005$). The PLC at each time point during and after radiation therapy was lower in patients receiving integrated RT than in those receiving hybrid RT ($P < .05$). Before and after propensity score matching, integrated RT was significantly associated with lower nadir PLC after adjusting for radiation therapy targets and age ($P < .001$).

Conclusions: Breast cancer patients had prolonged lymphopenia post−RT. Integrated RT increased the risk of RIL and adversely affected recovery. Therefore, an appropriate RT technique should be considered to minimize RIL.

Introduction

Postmastectomy radiation therapy (RT) could reduce breast cancer mortality rates.[1] Radiation exposure stimulates tumor antigen release and immune cell recruitment.[2] Meanwhile, it also causes immunosuppression by damaging immune cells, especially lymphocytes.[2,3] Circulating lymphocytes are highly radiosensitive,[4,5] and radiation-induced lymphopenia (RIL) is very common in RT-treated patients. RIL or peripheral lymphocyte count (PLC)-related parameters are negative prognostic factors for breast cancer[6,7] and other solid tumors.[8-12] Identifying RIL-associated risk factors is essential in developing interventions to prevent or mitigate the immunosuppressive effects of RT.

Few studies have explored RIL risk factors in breast cancer,[13,14] especially for patients receiving hypofractionated RT. Nowadays, integrated photon-based intensity modulated techniques, like multibeam intensity modulated RT (IMRT), volumetric modulated arc therapy (VMAT), and helical tomotherapy (HT), are widely used. However, they have the disadvantages of low-dose spread, and their impact on RIL remains unclear.

Recovery from RIL correlates with superior survival in several cancers.[15,16] Clarification of the dynamic changes in PLC might provide insights into the physiological response to RIL and lead to the development of strategies to promote recovery from RIL. However, little is known about when PLC recovery begins after RT and the time required for recovery in breast cancer patients. This study involved a prospective multicenter patient cohort and aimed to investigate longitudinal lymphocyte changes and RIL risk factors after postmastectomy hypofractionated RT among breast cancer patients, especially focusing on RT techniques.

Methods and Materials

Patient population

This pooled cohort study included patients from 2 prospective trials. Patients treated between October

2017 and January 2022 from a prospective cohort study (NCT04528225) and the POTENTIAL trial,[17] a multicenter prospective phase 3 randomized trial (NCT04320979), were included. The POTENTIAL trial aims to assess the efficacy and safety of internal mammary nodal irradiation (IMNI) in the context of modern systemic treatment and RT techniques. Participants enrolled in the trial will be randomly assigned in a 1:1 ratio to receive IMNI or not. Both study protocols were approved by our institutional review board (NCC2018S-116 and 19/317−2101). Overall, 1206 patients were identified. The eligibility criteria were as follows: histologically confirmed invasive breast cancer; mastectomy and axillary dissection; and irradiation with hypofractionated regimes in 15 fractions. Exclusion criteria were as follows: RT with conventional fractionations (n = 549) or 1-stage breast reconstruction (n = 30), synchronous bilateral breast cancer with bilateral RT (n = 3), and without PLC during RT (n = 17). Ultimately, 607 patients from 8 centers were included. Among them, 316 (59.5%) were from the prospective cohort study conducted between October 2017 and September 2020, whose data were used for this retrospective secondary analysis, and 291 (47.9%) patients were enrolled in the POTENTIAL trial initiated in May 2020. The purposes of the POTENTIAL trial included analyzing RIL as an adverse event. Thus, a prospectively planned analysis of RIL was performed for these patients preliminarily.

RT

CT-based 3D treatment plans were used, delivering a total dose of 43.5 Gy in 15 daily fractions. RT techniques included hybrid and integrated RT. Hybrid RT, which was frequently used until 2020, used electron fields for the chest wall and internal mammary node (IMN) region, with 2D technique, 3D conformal technique (3D CRT), IMRT, or VMAT for the supraclavicular and axillary nodal region. Integrated RT involved photon-based intensity modulated techniques including IMRT, VMAT, and HT for irradiation of all target volumes, which has been frequently used after 2020.

PLC and outcome assessment

PLCs were tested before RT (pre-RT PLC = pre-PLC), weekly during RT, at 3 and 6 months after RT, and then every 6 months. According to the POTENTIAL trial protocol, PLCs were also tested 1 and 2 weeks after RT. Nadir PLC during RT was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0.

Previous studies have highlighted the negative impact of lymphopenia on breast cancer prognosis[7,18-23] and confirmed that reduced PLC contributed to suboptimal outcomes. Thus, our study focused on nadir PLC during RT as the primary outcome and analyzed associated factors.

Statistical analysis

Continuous variables are summarized as mean ± standard deviation. Univariate analysis used independent *t* test, 1-way ANOVA, Kruskal-Wallis H test, and Spearman's correlation analysis. Correlations between continuous variables and PLC were evaluated using Spearman's correlation analysis. Relevant factors were assessed for multicollinearity using VIF.[24] Potential variables with $P <$ .25 in the univariate analysis and clinically relevant factors (laterality[7]) were included in a multivariable linear regression model. To further evaluate the association between the RT technique and RIL, a directed acyclic graph was used to explore potential confounders.[25,26] Paired *t* tests compared pre-PLC and PLCs at each time point after RT. Categorical variables are summarized as proportions and compared using χ^2 analysis or Fisher exact test. Propensity score matching (PSM) was used to minimize differences between the hybrid and integrated RT groups. PSM was performed after considering all possible relevant factors. The matching approach was 1:1 nearest neighbor matching within a caliper distance of 10%. The Bonferroni adjustment was used for multiple tests. A significance level of $P < .05$ (2-tailed) was considered statistically significant, while a *P* value of <.007 was used for comparing pre-PLC and PLCs at each time point after RT. Statistical analyses were performed using SPSS version 24.0 and R 4.1.1.

Results

Patient characteristics

Table 1 shows the characteristics of the 607 patients. All patients received a median of 8 (range, 4-16) chemotherapy cycles before RT, with a median of 6 (range, 1-13) cycles for neoadjuvant chemotherapy (22.6% of patients) and 8 cycles for adjuvant chemotherapy (85.5% of patients). No patients were treated with concurrent systemic agents during RT, and no patients received immunotherapy. All patients received irradiation to the chest wall and supra/infraclavicular fossa and axilla level II on the same slices. The IMN region was irradiated in 246 (40.5%) patients and axilla level I was irradiated in 51 (8.6%) patients. A total of 605 (99.7%) were treated with 43.5 Gy in 15 fractions, 1 (0.2%) patient with 40.5 Gy in

Table 1 Patient characteristics and univariate analysis for nadir PLC during radiation therapy

Characteristics	Total, n (%)	Nadir PLC	*P* value	*r*
Age (y), median (range)	49 (26-71)	-	.001	0.129
BMI (kg/m^2), median (range)	24.5 (16.7-35.6)	-	<.001	0.177
Laterality			.752	
Left	307 (50.6)	0.75 ± 0.32		-
Right	300 (49.4)	0.75 ± 0.34		-
Tumor location			.468	
Inner quadrants	182 (30.0)	0.73 ± 0.31		-
Other quadrants	422 (69.5)	0.76 ± 0.34		-
Unknown	3 (0.5)			
Lymphovascular invasion			.489	
No	175 (28.8)	0.75 ± 0.30		-
Yes	369 (60.8)	0.73 ± 0.35		-
Unknown	63 (10.4)			
Histological grade			.806	
I	21 (3.5)	0.76 ± 0.31		-
II	330 (54.4)	0.75 ± 0.36		-
III	218 (35.9)	0.74 ± 0.28		-
Unknown	38 (6.3)			
Stage			.044	
I-IIIA	453 (74.6)	0.77 ± 0.31		-
IIIB-IIIC	154 (25.4)	0.70 ± 0.38		-
Molecular subtype			.027	
Luminal A	75 (12.4)	0.74 ± 0.33		-
Luminal B-HER2 negative	285 (47.0)	0.71 ± 0.29		-
Luminal B-HER2 positive	111 (18.3)	0.79 ± 0.33		-
HER2 overexpression	68 (11.2)	0.82 ± 0.29		-
Triple negative	68 (11.2)	0.81 ± 0.47		-
Neoadjuvant chemotherapy			.437	
No	470 (77.4)	0.76 ± 0.34		-
Yes	137 (22.6)	0.73 ± 0.31		-
Adjuvant chemotherapy			.975	
No	88 (14.5)	0.75 ± 0.28		-
Yes	519 (85.5)	0.75 ± 0.34		-
Total cycles of chemotherapy			.960	
≤6	117 (19.3)	0.75 ± 0.29		-
>6	490 (80.7)	0.75 ± 0.34		-
Chemotherapy regimen			.009	
Anthracycline-based chemotherapy without taxane	7 (1.2)	0.94 ± 0.42		-
Taxane-based chemotherapy without anthracycline	24 (4.0)	0.95 ± 0.36		-
Anthracycline and taxane-based chemotherapy	512 (84.3)	0.73 ± 0.33		-
Taxane and platinum-based chemotherapy	49 (8.1)	0.76 ± 0.32		-

(*continued on next page*)

Table 1 (*Continued*)

Characteristics	Total, n (%)	Nadir PLC	*P* value	*r*
Other regimens without anthracycline or taxane	15 (2.5)	0.86 ± 0.33		-
Radiation therapy targets			<.001	
CW + SC	338 (55.7)	0.84 ± 0.36		-
CW + SC + IMN	217 (35.7)	0.64 ± 0.24		-
CW + SC + axilla	23 (3.8)	0.61 ± 0.28		-
CW + SC + IMN + axilla	29 (4.8)	0.62 ± 0.27		-
Radiation therapy technique			<.001	
Hybrid radiation therapy	338 (55.7)	0.87 ± 0.35		-
Integrated radiation therapy	269 (44.3)	0.60 ± 0.23		-
Pre-PLC ($\times 10^9$/L)	1.56 ± 0.48	-	<.001	0.464

Abbreviations: BMI = body mass index; CW = chest wall; HER2 = human epidermal growth factor receptor 2; IMN = internal mammary nodes; nadir PLC = the minimum peripheral lymphocyte count during radiation therapy; pre-PLC = peripheral lymphocyte counts prior to radiation therapy; SC = supraclavicular fossa.

15 fractions, and 1 (0.2%) patient with 40.05 Gy in 15 fractions.

Hybrid and integrated RT was used in 338 (55.7%) and 269 (44.3%) patients, respectively. Among those treated with hybrid RT, 2-D technique, 3D CRT, IMRT, and VMAT techniques were used to irradiate the supraclavicular and axillary nodal region in 76 (22.5%), 11 (3.3%), 181 (53.6%), and 70 (20.7%) patients, respectively. Among those treated with integrated RT, the VMAT technique was the most commonly used (n = 233, 86.6%), followed by IMRT (n = 35, 13.0%), and HT (n = 1, 0.4%).

Longitudinal analyses of PLC

The median follow-up period was 4.3 months (range, 0.6-16.1 months) for the patients in the POTENTIAL trial and 26.5 months (range, 0.2-54.8 months) for the prospective cohort. The pre-PLC was 1.56 ± 0.48 × 10^9/L and 8.6% of the patients had baseline lymphopenia: grade 1, 6.9%, and grade 2, 1.6%. During RT, nadir PLC significantly decreased to 0.75 ± 0.33 × 10^9/L ($P < .001$). Overall, 495 (81.5%) patients developed RIL. The incidence of grade 1, 2, 3, and 4 RIL was 18.0%, 43.7%, 19.4%, and 0.5%, respectively.

Figure 1A shows the PLC change among 291 patients in the POTENTIAL trial, the recruitment for which started in May 2020. During RT, PLCs declined steadily. The mean PLC dropped from 1.52 ± 0.50 to the nadir (0.58 ± 0.22 × 10^9/L) at week 3 during RT. PLCs started to recover and recovered to 0.64 ± 0.25 × 10^9/L at 1 week after RT, the number being significantly higher than that at week 3 during RT ($P = .002$).

The PLC change for all 607 patients is shown in Fig. 1B. PLCs dropped during RT and gradually increased thereafter. The PLCs remained low in the 6 months after RT compared with pre-PLCs ($P < .001$) and recovered to pre-RT levels at 1 year after RT (pre-PLCs vs 1 year after RT, 1.56 ± 0.48 vs 1.51 ± 0.43 × 10^9/L; $P = .037$). PLCs increased to 1.70 ± 0.45 × 10^9/L at 2 years after RT, which was significantly higher than the pre-PLC ($P < .001$), and remained stable thereafter.

Risk factors associated with RIL

Univariate analysis revealed that age, body mass index (BMI), disease stage, molecular subtype, chemotherapy regimen, RT targets, RT technique, and pre-PLC were potential factors associated with the nadir PLC during RT ($P < .05$) (Table 1). Because laterality has been reported to be associated with RIL,[7] we also analyzed laterality as a covariate. Variables with a *P* value of <.25 in the univariate analysis and laterality were assessed for multicollinearity (Table E1). No variable with VIF >10 was found, indicating that there was no collinearity between the variables.[24]

Multivariable linear regression analysis showed that young age, low BMI, multiregion RT targets, integrated RT, and low pre-PLC were independent risk factors influencing nadir PLC ($P < .005$) (Table 2). Pre-PLC showed a positive correlation with nadir PLC during RT (r = 0.459, $P < .001$) (Fig. 2).

The effect of RT techniques on RIL

Patients who received integrated RT had a significantly lower nadir PLC (0.60 ± 0.23 × 10^9/L) compared to those who received hybrid RT (0.87 ± 0.35 × 10^9/L, $P<.001$)

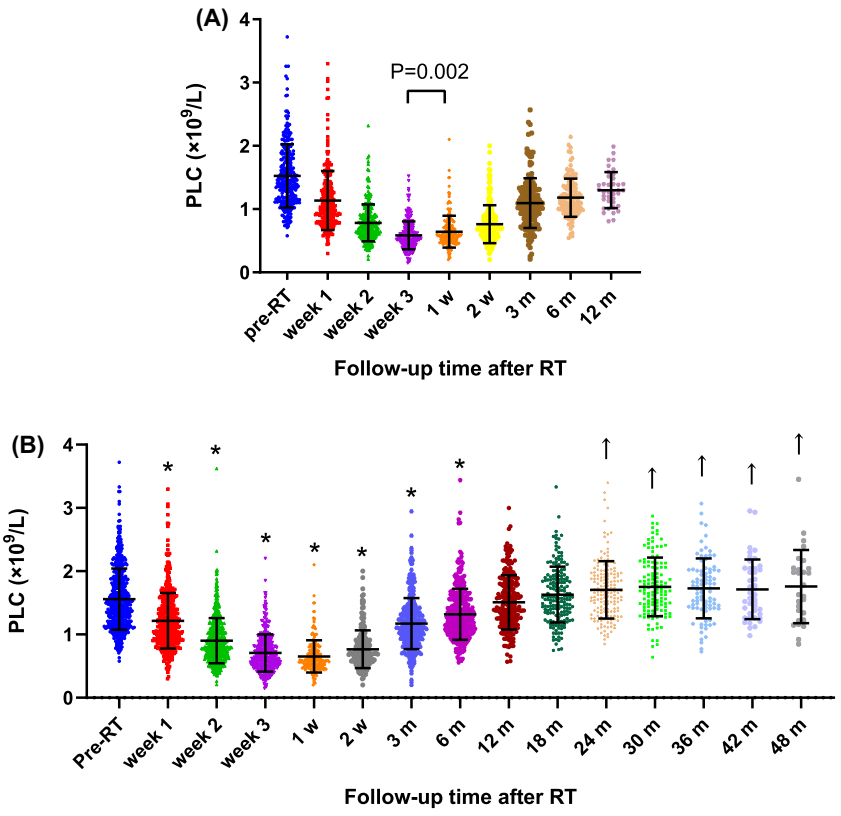

Figure 1 Dynamic changes in the peripheral lymphocyte counts (PLCs) among (A) patients who were enrolled in the POTENTIAL trial (N = 291) and (B) the entire cohort (N = 607). The error bars represent standard deviation values. The *P* values in Fig. 1B represent the comparison of PLCs at each time point after RT with pre-RT PLC (pre-PLC). *The PLC was significantly lower than the pre-PLC ($P < .007$). ↑The PLC was significantly higher than the pre-PLC ($P < .007$).
Abbreviations: m = month(s); pre-RT = prior to radiation therapy; w = week(s).

(Table 1). Dynamic changes in PLC over time revealed similar trends for integrated and hybrid RT (Fig. 3). However, integrated RT induced more serious RIL and resulted in slower recovery compared with the hybrid RT group (Fig. 3).

A directed acyclic graph (Fig. E1) was generated to identify confounding variables requiring adjustment, which included RT targets and age.[26] The multivariable regression model including RT technique, RT targets, and age showed a significant association between RT

Table 2 Multivariable linear regression analysis of nadir PLC

Variable	Estimate	Standardized beta coefficients	*P* value
Age (y)	0.004	0.116	<.001
BMI (kg/m^2)	0.010	0.098	.003
Laterality	−0.014	−0.021	.514
Stage	−0.000	−0.000	.988
Molecular subtype	0.011	0.038	.239
Chemotherapy regimen	−0.007	−0.014	.655
Radiation therapy targets	−0.056	−0.131	<.001
Radiation therapy technique	−0.236	−0.355	<.001
Pre-PLC	0.298	0.435	<.001

Abbreviations: BMI = body mass index; nadir PLC = the minimum peripheral lymphocyte count during radiation therapy; pre-PLC = peripheral lymphocyte counts prior to radiation therapy.

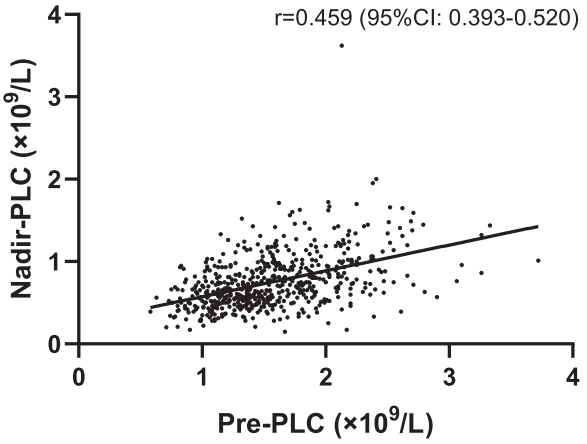

Figure 2 Relationship between peripheral lymphocyte counts prior to radiation therapy (RT) and nadir peripheral lymphocyte counts during RT.
Abbreviations: nadir PLC = minimum peripheral lymphocyte count during RT; pre-PLC = peripheral lymphocyte count prior to RT.

technique and nadir PLC (estimate = −0.236; standardized beta coefficients = −0.355; $P < .001$).

The difference in RT targets mainly lies in whether IMN irradiation (IMNI) was administered. The nadir PLC in 246 (41.0%) patients who received IMNI was significantly lower than that in the 361 (59.5%) patients who did not receive IMNI (0.63 ± 0.24 vs $0.83 \pm 0.36 \times 10^9$/L, $P < .001$). More patients with IMNI received integrated RT compared with those without IMNI (56.9% vs 35.7%, $P < .001$). Therefore, We further analyzed the interaction between IMNI and RT techniques with nadir PLC (Table 3). Both IMNI and integrated RT were independent risk factors for RIL, with a statistically significant interaction between IMNI and RT techniques (P for interaction < .001). Patients were divided into subgroups based on RT techniques and whether they received IMNI and pairwise comparisons were performed. The nadir PLC in patients receiving both IMNI and integrated RT was the lowest ($0.57 \pm 0.20 \times 10^9$/L). Whether IMNI was administered or not, significant differences in nadir PLC were observed between different RT techniques (Bonferroni adjusted P value < .001). However, the effects of IMNI on RIL vary across the subgroups of different RT techniques. Among patients receiving hybrid RT, a significant difference was observed in nadir PLC between no IMNI and IMNI subgroup (Bonferroni adjusted P value < .001). However, among patients receiving integrated RT, no significant differences in nadir PLC were found between the 2 subgroups (Bonferroni adjusted P value = .463).

The distribution of patients treated with integrated and hybrid RT in the entire cohort is shown in Table E2. After PSM, a subset of 344 patients (172 matched pairs) was chosen for the analyses. RT technique remained significantly associated with nadir PLC after adjustment of RT targets and age (estimate = −0.229; standardized beta coefficients = −0.342; $P < .001$).

Furthermore, among patients treated with hybrid RT, the nadir PLC during RT was significantly higher in those treated with 2-D technique or 3D CRT compared with VMAT or IMRT (0.99 ± 0.34 vs $0.83 \pm 0.34 \times 10^9$/L, $P < .001$).

Discussion

In this study, we analyzed 607 breast cancer patients from prospective cohorts and found that grade ≥3 RIL developed in 19.9% during RT and the nadir PLC was

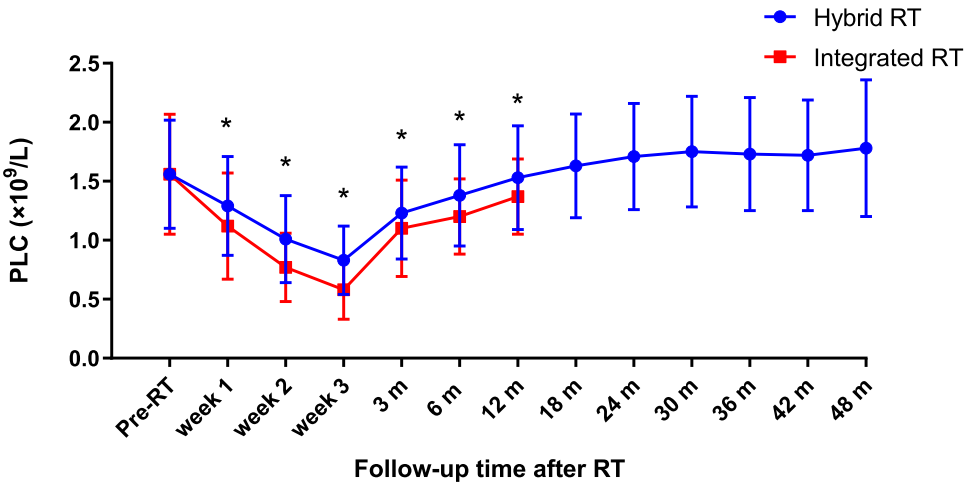

Figure 3 Peripheral lymphocyte counts (PLCs) over time in the integrated and hybrid radiation therapy (RT) groups. *The PLC in the integrated RT group was significantly lower than that in the hybrid RT group ($P < .05$).
Abbreviations: m = month(s); pre-RT = prior to RT; w = week(s).

Table 3 Evaluation of interactions between IMN irradiation and radiation therapy techniques with nadir PLC

Variable	N (%)	Nadir PLC	*P* value
IMN irradiation			<.001
Radiation therapy technique			<.001
IMN irradiation *Radiation therapy technique			<.001
No IMNI + hybrid radiation therapy	232 (38.2)	0.94 ± 0.36	
IMNI + hybrid radiation therapy	106 (17.5)	0.72 ± 0.26	
No IMNI + integrated radiation therapy	129 (21.3)	0.63 ± 0.26	
IMNI + integrated radiation therapy	140 (23.1)	0.57 ± 0.20	

Abbreviations: IMNI = internal mammary node; IMNI = internal mammary nodal irradiation; nadir PLC = the minimum peripheral lymphocyte count during radiation therapy.
Analyses were adjusted for age, body mass index, laterality, stage, molecular subtype, chemotherapy regimen, and peripheral lymphocyte counts prior to radiation therapy.

$0.75 \pm 0.33 \times 10^9$/L. To our knowledge, this is the first longitudinal analysis of long-term PLC changes after post-mastectomy hypofractionated RT. PLCs started to recover 1 week after RT, reached pre-RT levels 1 year after RT, and higher levels 2 years after RT. Young age, low BMI, multiregion RT targets, integrated RT techniques, and pre-RT PLC were independent risk factors influencing nadir PLC. Integrated RT induced more serious RIL and resulted in slower recovery compared to hybrid RT.

Limited data exist on post-RT recovery. We found PLCs started to recover 1 week after RT. A post hoc analysis of 598 patients from a phase 3 randomized clinical trial comparing hypofractionated RT (43.5 Gy in 15 fractions over 3 weeks) to conventional fractionated RT (50 Gy in 25 fractions over 5 weeks) found similar nadir PLCs during the initial 3 weeks of RT. However, the nadir PLC of the last 2 weeks in the conventional fractionated RT group was significantly lower than that in the initial 3 weeks, suggesting a continuous PLC decrease in the fourth and fifth weeks.[7] Our study shows that PLC recovery may be earlier in patients receiving hypofractionated RT compared to conventional fractionated RT. Besides, we observed that lymphopenia lasted over 6 months after RT. The PLCs recovered to pre-RT levels at 1 year and reached higher levels at 2 years. Because all patients received surgery and chemotherapy before RT, the higher PLC level at 2 years after RT, rather than pre-PLC, might be the normal baseline level. Previous studies in patients with other solid tumors showed persistent lymphopenia until 12 months after RT,[12,27,28] consistent with our findings. Another study showed PLCs were significantly lower in all breast cancer patients (n = 40) before and 1 year after RT than in healthy controls (n = 20).[29] Prolonged lymphopenia may lead to additional immunosuppression for months.

Given that 19.9% of our patients developed grade ≥3 RIL during RT, which may impede antitumor immunity,[12,30-33] identifying the risk factors for RIL is important. We demonstrated that young age, low BMI, multiregion RT targets, integrated RT technique, and low pre-PLC were independent risk factors influencing nadir PLC. Possible risk factors for RIL in patients with various solid tumors included treatment factors, such as large target volume size[15,34-37]; multiple radiation sites[38]; conventional fractionated RT or twice-daily radiation fractionation[8,35,39]; long overall RT time[39-41]; photon beam RT (vs proton beam RT)[36,37,39]; high dosimetric parameters of normal tissues[35,40-45] and chemotherapy,[35,38] and patient/tumor factors, including older age,[36,37,45] XRCC1 rs25487 genotypes,[45] advanced disease stage,[35] tumor location in the pancreatic body or tail (vs head),[15] and low baseline PLC.[15,37,40,41,44] Our findings confirmed the association between multiregion RT targets and RIL, consistent with previous studies in patients with solid malignant tumors.[38] Large target volume will lead to more depletion of circulating lymphocytes in the bloodstream and increase the risk of RIL. In our cohort, all patients underwent axillary lymph node dissection. Irradiation of the level I axilla was performed only in those at high risk of axillary nodal recurrence, in order to minimize the risk of treatment-related lymphedema.[46] IMNI in the POTENTIAL trial was administered based on randomization. In fact, with no evidence of overall survival benefits and toxicity concerns, especially in the context of modern systemic therapy, the adoption of prophylactic IMNI varied in routine clinical practice.[47] The disadvantages of the hybrid technique with regard to lung and heart doses may become more apparent if all nodes were being treated. Studies of RIL in breast cancer patients are limited.[7,13,14] Reported risk factors included RT technique involving VMAT (vs 2-D technique or 3D CRT), high mean lung dose, chemotherapy, right-sided tumors, low BMI, and a low pre-RT PLC.[7,13,14] However, the timing of RIL determination and thresholds for defining RIL differ across studies. In our study, younger age and lower BMI were independent

factors associated with lower nadir PLC. The higher likelihood of RIL in patients with low BMI can be explained by the smaller total blood volume and lymphocyte reserve.[7] Few studies have analyzed the correlations between age and nadir PLC during RT as continuous variables. We speculate that the lymphocytes of younger patients are more sensitive to RT compared with those of older patients. Such hypotheses and the effect of age on RIL need further research.

In this study, integrated RT significantly decreased nadir PLC compared with hybrid RT, potentially due to increased volumes of low-dose irradiation. Hybrid RT with electron irradiation to irradiate the chest wall minimized low-dose volume for adjacent organs such as lungs, heart, and liver, which harbor large lymphocyte pools. A recent study showed VMAT treatment in breast cancer patients had greater PLC reduction and lung V5 compared to 3D CRT.[13] Lymphocytes and their precursors are extremely radiosensitive,[5] and exposure to low-dose irradiation may increase lymphocyte destruction. Several studies indicate that lower doses rather than higher doses are more strongly correlated with RIL risk, illustrating the clinical significance of low-dose irradiation.[35,42,45,48] Further research is required to evaluate the impact of integrated RT techniques on prognosis. Hybrid RT can be a good option to reduce RIL risk, as long as it meets the dosimetric requirements of the tumor target and organ-at-risk. The 5-year locoregional recurrence rate was only 8.1% to 8.3% after receiving hybrid RT without IMNI among 820 patients with 4 or more positive axillary lymph nodes or stage T3 to T4 disease after mastectomy in a randomized phase 3 trial.[49] Integrated RT may be reserved for challenging situations with its advantages in adequate target volume coverage, dose uniformity, and target conformity. For example, integrated RT is preferred when the IMNs are too deep or the chest wall is too thick to be irradiated adequately by hybrid RT. In addition, IMRT/VMAT is typically used when hybrid RT fails to adequately achieve planning goals with regard to other organs at risk, such as the lung and heart. It is important to note that the somewhat higher risk of RIL may not be enough to offset the other toxicities that may be higher with a hybrid RT plan. To summarize, the tradeoffs between RIL risk and superior dose distribution with integrated RT compared with hybrid RT should be considered during technique choice. Reducing the risk of RIL without compromising target coverage may benefit disease outcomes.

Our findings confirmed the significance of the pre-PLC for predicting RIL, consistent with the common acknowledgment. One possible physiological explanation is that baseline PLC reflects a patient's physical condition and immunity reserve capacity. Pre-PLC may also indicate tumor-induced immunosuppression driving tumor progression.[50-52]

Our study had limitations. First, the short follow-up time and incomplete survival data require long-term follow-up to assess specific PLC-related parameters with prognostic value and the effect of RT techniques on survival. Second, analysis of dosimetric factors is necessary to understand why different techniques affect RIL risk. More extensive dosimetric studies are ongoing to evaluate the causes of the different RIL risks between the different RT techniques and to develop a prediction model of RIL. Third, missing data on PLCs at some time points during and after RT may introduce bias. Fourth, mechanisms of RIL development and regulation are desired for developing strategies to facilitate early PLC recovery. The ongoing translational substudy of the POTENTIAL trial, which includes the collection of lymphocyte subpopulations and blood samples for cytokine detection, will provide valuable insights into the immunology underlying RIL. Despite limitations, our study demonstrated longitudinal PLC changes and the negative effect of advanced RT techniques on RIL in prospective cohorts with a consistent treatment paradigm and structured follow-up. We will further perform a dosimetric analysis and examine dosimetric factors associated with RIL. Radiation doses to bone marrow, lymphoid organs (eg, spleen, thymus), and organs with abundant blood circulation (eg, heart, lungs, body) were demonstrated to be associated with RIL in different solid tumors. Recently, various RIL prediction models have been established based on different definitions of RIL and different modeling frameworks.[53] None of the proposed models addressed both RIL severity and duration. However, at present, there is still no clear picture of how to make radiation dose constraints to prevent the risk of RIL during and after RT. To strengthen RT personalization, it is also essential to develop modeling frameworks incorporating the individual's sensitivity to radiation.[53] Identifying dose constraints for the organs most likely implicated in RIL development will help reduce the RIL risk when delivering integrated RT. In the future, we will take advantage of the multifaceted information provided by each of the current approaches and build an accurate RIL prediction model that can be used as an applicable tool in daily clinical practice.

Conclusion

Our study demonstrated breast cancer patients had prolonged lymphopenia after RT. Integrated advanced RT increased RIL risk and adversely affected recovery. Therefore, appropriate RT technique selection is necessary to minimize radiation toxicity to the immune system.

Disclosures

None.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.adro.2025.101750.

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