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Systemic Inflammation After Radiation Predicts Locoregional Recurrence, Progression, and Mortality in Stage II-III Triple-Negative Breast Cancer

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Abstract

Purpose: Patients with triple-negative breast cancer experience high rates of recurrence after radiation, which may be facilitated by the recruitment of circulating tumor cells to proinflammatory microenvironments in the absence of lymphocytes. We hypothesized that patients with lymphopenia and elevated inflammatory hematologic markers after radiation therapy would have an increased risk of locoregional failure.

Methods and Materials: With approval, we retrospectively studied a cohort of women treated with adjuvant radiation therapy for stage II-III triple-negative breast cancer. We analyzed the relationship between post—radiation therapy neutrophil:lymphocyte ratio (NLR) and locoregional recurrence by using Cox regression.

Results: One-hundred thirty patients met inclusion criteria, and median follow-up time was 7.6 years. Patients with an NLR ≥3 had a higher rate of locoregional failure ($P = .04$) and lower overall survival ($P = .04$). After adjusting for stage (hazard ratio [HR], 5.5; $P < .0001$) and neoadjuvant chemotherapy (HR, 2.5; $P = .0162$), NLR was highly predictive of locoregional failure (HR, 1.4; $P = .0009$). NLR was also highly predictive of overall survival (HR, 1.3; $P = .0007$) after adjustment for stage and neoadjuvant chemotherapy.

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Conclusions: Innate peripheral inflammation after radiation therapy for triple-negative breast cancer in an immunocompromised setting may be a novel prognostic biomarker for locoregional recurrence, progression, and survival. This finding supports preclinical studies of post—radiation therapy inflammation-mediated tumor progression. Further studies are needed to confirm this finding and develop treatment strategies.

Summary

Preclinical data suggest that triple-negative breast cancer may recur in irradiated tissue as a result of stromal-based attraction of circulating tumor cells into a proinflammatory, lymphocyte-depleted microenvironment. We hypothesized that patients with triple-negative breast cancer are at risk for recurrence based on the inflammatory response to radiation. We studied 130 patients with stage II-III triple-negative breast cancer. We found that elevated neutrophil:lymphocyte ratio after radiation is an independent predictor of locoregional relapse, progression-free survival, and overall survival.

Introduction

Radiation therapy (RT) invokes an immunogenic process by which DNA damage in the tumor cell facilitates the presentation of tumor neoantigens to infiltrating effector lymphocytes for immunorecognition of tumor cells and tumor cell destruction.[1] While lymphocytes contribute to the tumor-killing mechanism of RT, lymphocytes are highly radiosensitive.[2] Even in the absence of cytotoxic chemotherapy, lymphopenia is common after RT and has been associated with recurrence and mortality in several solid tumors.[3] In breast cancer, however, very few studies have examined the relationship between post-RT lymphopenia and outcome. In triple-negative breast cancer (TNBC), lymphocyte-mediated tumor cell death after RT may be even more crucial than in other subtypes with lower mutational burden.[4-7] Because mutational burden is thought to correlate with neoantigen load, the probability of TNBC eradication by immune-mediated tumor cell killing may be influenced significantly by lymphocyte activity, viability, and abundance.[4,5,8] Thus, in addition to the poor prognosis of TNBC, a substantial impetus exists to correct the dearth of evidence regarding post-RT lymphopenia and TNBC outcomes.[9]

Recently, a clinical correlation was reported between postdiagnosis lymphopenia and prognosis.[10] More recently, a mouse model of lymphopenia was used to show that irradiation of normal breast tissue leads to the recruitment of macrophages through stromal chemokine secretion.[11] In this model, these infiltrating macrophages were shown to secrete additional chemokines that attracted circulating TNBC cells, which subsequently invaded the irradiated stroma.[11] This finding suggested that TNBC may recur through the creation of irradiated tumor niches by a proinflammatory, lymphocyte-depleted microenvironment.

Although pretreatment inflammation, commonly measured as neutrophil:lymphocyte ratio (NLR), has been correlated with prognosis in breast cancer, very few groups have reported on the prognostic implications of dynamic inflammatory changes after RT.[12-16] Furthermore, the relationships between treatment, such as RT, and systemic inflammatory dynamics remain poorly understood; how these changes alter prognosis is also unknown.

To explore the preclinical hypothesis that an irradiated, proinflammatory, and immunocompromised microenvironment leads to TNBC recurrence, we examined the relationship between patient outcomes and the systemic inflammatory response to RT as a surrogate for the microenvironment. Thus, the purpose of our study was to examine the relationship between RT and hematologic markers of inflammation and immunity and to investigate whether time-varying markers of inflammation and immunity after RT were predictive of patient outcomes.

Methods and Materials

Patient population

After obtaining institutional review board approval from each institution, we performed a multi-institutional retrospective cohort study of patients seen between 1999 and 2012 with stage II-III TNBC who were treated with definitive RT. Electronic medical records were compiled by the Vanderbilt Research Derivative and the Stanford Cancer Institute Research Database.[17] The Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) criteria were used to guide the design and reporting of this study.

Inclusion and exclusion criteria

Female patients who were at least 18 years of age with histologically confirmed, invasive, stage II-III TNBC treated with definitive external beam RT were included. Chemotherapy, RT, and surgery were chosen in a non-randomized manner according to standard of care practice with multidisciplinary tumor board input. TNBC status was defined as <1% immunohistochemical staining for estrogen receptor and progesterone receptor and no amplification or overexpression of human epidermal growth factor receptor 2. Patients with either neoadjuvant or adjuvant chemotherapy, or both, were included. Patients were excluded if they had hematopoietic or bone marrow infiltration disorders. Patients with a history of malignancy were eligible provided there was no evidence of disease at the time of TNBC diagnosis and treatment. Patients were followed for a minimum of 5 years from the end of RT. Patients were required to have at least 1 peripheral complete blood count (CBC) with differential during RT or within 1 year after RT completion based on preclinical evidence of the importance of prolonged inflammation and immunosuppression after RT for TNBC.[11] Peripheral blood was obtained, preserved, stored, and analyzed by Clinical Laboratory Improvements Amendments (CLIA) certified laboratories, including at Vanderbilt University Medical Center and Stanford University Medical Center, according to standard protocols using automated flow cytometry. Between 0.5 and 2 mL of whole blood were collected in ethylenediaminetetraacetic acid—containing tubes. All assays were blinded to the study endpoint and obtained outside the context of the study as part of standard of care.

Study predictors

Data were abstracted from the medical record until April 1, 2019. The highest combined histologic grade was recorded. Pretreatment clinical stage was used for patients who underwent neoadjuvant chemotherapy, and the remaining patients were pathologically staged at the time of surgery. Staging was based on the American Joint Committee on

Cancer Staging Manual, eightth edition. The presence or absence of lymphovascular invasion (LVI) was recorded at the time of surgery.

Because prior studies suggested prognostic significance of prolonged lymphopenia after RT, every CBC available from the start of RT through 1 year after RT was included in the data to investigate the effects of the pattern of markers over time on prognosis.[3,11] From each CBC, platelet count, absolute neutrophil count, absolute lymphocyte count (ALC), and absolute monocyte count were recorded. NLR, platelet: lymphocyte ratio (PLR), and monocyte:lymphocyte ratio (MLR) were then calculated for each CBC. The mean of each hematologic parameter was obtained for each patient.

Outcome measures

The primary study outcome was time to locoregional failure (LRF). Secondary study outcomes were time to progression-free survival (PFS) and time to overall survival (OS). LRF was defined as the time from start of RT until any radiologic or pathologic evidence of disease in the ipsilateral breast/chest wall or ipsilateral regional nodes, with death being considered a competing event for LRF. Distant failure was not considered a competing event for LRF. Time to PFS was defined as time from start of RT until clinically or radiologically suspected local, regional, or distant failure or death, whichever came first. Patients who did not experience any local, regional, or distant failures or death were censored at the date of last follow-up. Time to OS was defined as the time from the start of RT until death from any cause. The sample size was determined by including all patients meeting inclusion and exclusion criteria to maximize statistical power in the multivariable analysis.

Statistical analysis

The time-to-event outcome of LRF was analyzed using competing events analysis, with death as the competing event. The time-to-event outcomes of PFS and OS were analyzed using Kaplan-Meier curves, and medians with 95% confidence interval were calculated using Greenwood's formula. In these analyses the mean laboratory values per patient were used, and these predictors were dichotomized based on the median. Single-predictor Cox models with time-varying laboratory values were also evaluated. Multivariable Cox models with parsimonious predictor selection were performed to test the hypothesis that time-varying laboratory values contributed meaningful prognostic information for salient clinical predictors in the context of other known prognostic factors. The most optimal models were statistically selected by the score selection method using the branch-and-bound algorithm of Furnival and Wilson; in this approach, the highest likelihood score (χ^2) for all possible models was used to build unbiased models so that only variables that contributed significant and unique prognostic information were included.[18] For sufficient power and unbiased estimates, each model was required to have at least 10 events per predictor included. Time-to-event outcomes were constrained from the time of first laboratory measurement to the time of event or censor. All tests were 2-sided with an alpha level of 0.05. All analyses were performed using SAS v9.4 (SAS Institute Inc, Cary, NC) and all plots were generated using Prism v8.1 (GraphPad Software, La Jolla, CA).

Results

Patient characteristics

A total of 130 patients were enrolled (Table 1), including 53 patients treated at Stanford University Medical Center and 77 patients treated at Vanderbilt University Medical Center. Most patients had stage II disease (67%). Almost all patients received chemotherapy (97%), including neoadjuvant chemotherapy in 51% of patients and adjuvant chemotherapy in 72% of patients. After neoadjuvant chemotherapy, 17 (35%) patients had a complete pathologic response at the time of surgery. Most chemotherapy regimens included a combination of anthracyclines, taxanes, and cyclophosphamide. The most common radiation dose to the tumor bed was 60.4 Gy (38%) delivered over 33 fractions, including boost to the surgical scar, and 73% of patients received regional nodal irradiation. Each patient had a median of 4 laboratory measurements in the year after RT (range, 2-40). From the start of RT to 2 months after the conclusion of RT, 112 patients (86%) had laboratory measurements; from 2 months post-RT to 6 months post-RT, 84 patients (64%) had laboratory measurements; and from 6 months post-RT to 12 months post-RT, 101 patients (78%) had laboratory measurements. The overall median ALC was 1.08 K/μL (interquartile range [IQR], 0.79-1.40), the median NLR was 2.99 (IQR, 2.14-4.31), the median PLR was 216 (IQR, 159-286), and the median MLR was 0.40 (IQR, 0.28-0.61). There was no single predictor that correlated with mean laboratory values and clinicopathologic factors, including age, radiation dose, radiation fractions, neoadjuvant chemotherapy, adjuvant chemotherapy, stage, surgery, menopausal status, or nodal irradiation.

At a median follow-up time of 7.6 years (IQR, 0.5-19.3), a total of 34 (26%) deaths were observed during the study period, 31 of which were attributable to TNBC. Relapse occurred in 49 patients (38%). Isolated locoregional recurrence occurred in 9 (7%) patients, and 23 (18%) patients developed both locoregional and distant metastatic disease. The median time to any recurrence was 1.2 years (range, 0.25-10.25) after RT. Mortality occurred at a median time of 2 years after RT (range, 0.6-14). At 5 years after RT, OS was 78%, PFS was 67%, and LRF was 21%.

In single-predictor analysis, the following predictors were significantly correlated with LRF: neoadjuvant chemotherapy (hazard ratio [HR], 2.7; $P = .009$), adjuvant chemotherapy (HR, 0.2; $P < .0001$), LVI (HR, 2.4; $P = .01$), and stage (HR, 5.1; $P < .0001$) (see Table E1, available online at https://doi.org/10.1016/j.ijrobp.2019.11.398). Neoadjuvant chemotherapy (HR, 2.1; $P = .01$), adjuvant chemotherapy (HR, 0.37; $P = .001$), LVI (HR, 2.3; $P = .003$), stage (HR, 4.6; $P < .0001$), mastectomy versus lumpectomy (HR, 2.2; $P = .006$), and pathologic complete response (HR, 0.26; $P = .03$) were correlated with PFS. OS was correlated with neoadjuvant chemotherapy (HR, 2.5; $P = .01$), adjuvant chemotherapy (HR, 0.43; $P = .02$), stage (HR, 4.0; $P < .0001$), mastectomy versus lumpectomy (HR, 2.6; $P = .006$), and pathologic complete response (HR, 0.21; $P = .03$). With the exception of mastectomy versus lumpectomy and pathologic complete response, all of these predictors were significantly correlated with all three time-to-event outcomes. LRF was not significantly correlated with mastectomy versus lumpectomy (HR, 1.6; $P = .2$) or pathologic complete response (HR, 0.28; $P = .09$).

Single-predictor biomarker prognostic analysis

The relationships between postradiation systemic markers of inflammation and immunity and LRF, PFS, and OS are reported in Table 2. Elevated NLR as a continuous variable after RT was predictive of LRF (HR, 1.37; $P = .0029$), PFS (HR, 1.32; $P = .0012$), and OS (HR, 1.21; $P = .0054$). These results were consistent with the Kaplan-Meier analysis, which showed that a mean NLR ≥3 after RT was predictive of a higher cumulative incidence of LRF ($P = .04$) and lower OS ($P = .04$) (Fig. 1A-C). In the single-predictor Cox model, continuous MLR was significantly correlated with OS (HR, 3.8; $P = .0196$) but not with LRF (HR, 2.8; $P = .0872$) or PFS (HR, 2.1; $P = .4130$). In the Kaplan-Meier analysis, a mean MLR value ≥0.40 was correlated with lower survival ($P = .01$) (Fig. 1D). However, this mean MLR threshold was not significant for LRF ($P = .3589$) or PFS ($P = .0913$). Patients with higher continuous lymphocyte counts after RT had a lower cumulative incidence of LRF (HR, 0.40; $P = .0189$) and a higher rate of PFS (HR, 0.52; $P = .0280$). However, ALC was not associated with OS (HR, 0.53; $P = .0734$). The Kaplan-Meier analysis did not show any mean ALC threshold predictive of any study outcome.

Multivariable Cox analysis

The following predictors were submitted for evaluation for inclusion in each multivariable model: each time-varying laboratory measurement, age, body mass index, clinical T stage, clinical N stage, overall stage, receipt of neoadjuvant chemotherapy, pathologic complete response, histologic grade, mastectomy versus lumpectomy, lymphovascular invasion, and menopausal status. For each study outcome, the following predictors were consistently retained by multivariable optimized predictor selection: NLR, stage, adjuvant chemotherapy, neoadjuvant chemotherapy, and mastectomy versus lumpectomy. Only the PFS model had sufficient statistical power to include 5 predictors in a single model. In this 5-predictor model, stage (HR, 3.8; $P < .0001$), NLR (HR, 1.3; $P = .004$), and adjuvant chemotherapy (HR, 0.41; $P = .01$) retained statistical significance, but mastectomy versus lumpectomy (HR, 1.6; $P = .1$) and complete pathologic response (HR, 0.27; $P = .08$) did not (Table E2, available online at https://doi.org/10.1016/j.ijrobp.2019.11.398). Therefore, the most optimal PFS models included 3 predictors only; owing to power, the LRF and OS models also included 3 predictors.

For each study outcome, there were 3 consistent models. All models included NLR, stage, and 1 of the following predictors: mastectomy versus lumpectomy, adjuvant chemotherapy, or neoadjuvant chemotherapy (Table 3). For LRF, mastectomy versus lumpectomy was not significant (HR, 1.32; $P = .4621$), and for OS, adjuvant chemotherapy was not significant (HR, 0.52; $P = .0836$). Thus, the final model for LRF, PFS, and OS consisted of NLR, stage, and neoadjuvant chemotherapy. Compared with stage II disease, stage III disease was associated with a greater hazard of LRF (HR, 5.53; $P < .0001$), PFS (HR, 4.81; $P < .0001$), and dying (HR, 4.46; $P < .0001$). A 1 unit increase in the value of NLR (ie, a change from an NLR of 3 to 4) was associated with a greater hazard of LRF (HR, 1.40; $P = .0009$), PFS (HR, 1.35; $P = .0004$), and dying (HR, 1.27; $P = .0007$). Receiving neoadjuvant chemotherapy was associated with a greater hazard of LRF (HR, 2.53; $P = .0162$), PFS (HR, 2.02; $P = .0241$), and dying (HR, 2.32; $P = .0271$).

A cumulative incidence model was fit with LRF as the outcome and death as a competing risk, demonstrating a difference ($P < .0001$) among 4 cohorts stratified by stage and mean postradiation NLR: NLR <3 and stage II, NLR ≥3 and stage II, NLR <3 and stage III, and NLR ≥3 and stage III (Fig. 2). As suggested by the magnitude of the HR of the Cox multivariable analysis, stage is the strongest predictor of LRF, and the 2 groups with stage II have lower rates of LRF than the 2 groups with stage III. Within each stage, patients who have NLR <3 have a lower rate of LRF compared with patients with NLR ≥3.

Discussion

In this retrospective study of TNBC, systemic inflammation and immunocompromised status after RT were independently predictive of LRF, PFS, and OS. These clinical findings support the preclinical hypothesis that TNBC recurrence is mediated in part by a proinflammatory, lymphocyte-poor microenvironment within irradiated stroma. This study also suggests that systemic inflammatory measurements, such as NLR, PLR, and MLR, may be valuable prognostic biomarkers after RT. In addition to prognostic information, these biomarkers may provide an avenue to clinically model the characteristics of the irradiated microenvironment in real time.

The host adaptive immune response is thought to recognize RT-induced tumor neoantigen presentation and subsequently enhance RT-related tumor killing.[19] This notion has been supported by the relationship between intratumoral lymphocytes and prognosis.[20] Although RT promotes antitumor immunostimulation, normal tissue damage also activates the innate inflammatory response.[21,22] Although RT damage leading to neutrophil and macrophage infiltration signals for cleanup of necrotic debris within the microenvironment, the relationship between tumor cells and the innate immune system is controversial.[23-25] In fact, there is some evidence that the inflammatory response to RT may inadvertently promote TNBC progression locally and even distantly, and this hypothesis has been postulated in other cancers as well.[16,26-28]

In support of the hypothesis that RT promotes systemic inflammation and immunosuppression, resulting in a microenvironment conducive to TNBC recurrence, we find that an elevated NLR after RT strongly and independently predicts LRF, PFS, and OS. The prognostic significance demonstrated by this finding advances the concept of protumor neutrophil phenotypes induced by RT in the tumor microenvironment. In the irradiated microenvironment, such protumor neutrophils may directly promote immunosuppression by regulating infiltrating effector lymphocytes through mediators such as reactive oxygen species, nitric oxide, and arginase.[29-31] Radiation-driven neutrophil-mediated immunosuppression, in addition to radiation-induced lymphopenia, may therefore facilitate tumor escape via immunodetection avoidance.[11]

Interestingly, MLR did not provide direct prognostic information on LRF or PFS but instead on OS. The utility of MLR for predicting local failure may be challenged by the dichotomy of pro- and antitumor macrophage polarization states, differences between macrophages derived from circulating monocytes and resident tissue macrophages, and microenvironmental dynamics between tumor-associated macrophages, tumor, and

infiltrating monocytes, with nuances that are poorly evaluated by peripheral monocyte count.[11-32-34]

This work highlights several critical areas for further study. Determining the influence of host genetics, environmental factors, and tumor composition, among other factors, on patient response to RT may allow for more precise prediction of outcome before treatment. Such precision medicine techniques may even offer a clinical platform for tailoring TNBC therapies based on the predicted inflammatory and immunologic response to RT. In the era of checkpoint inhibitors, the significance of immunologically based predictive biomarkers may be even more relevant.[35] Although immunotherapy is not currently the standard of care for localized TNBC, adjuvant locoregional TNBC treatment paradigms may shift to include immunotherapy concurrently or in sequence with RT based on positive results from the metastatic literature. For example, the phase 3 IMpassion 130 trial recently reported that programmed death ligand 1 inhibition plus chemotherapy in untreated metastatic TNBC provided a PFS benefit enriched in programmed death ligand 1—positive tumors.[36] In light of such promising data, an increased understanding of the underlying mechanisms behind the clinical findings reported in our study may provide a basis for personalized strategies to minimize the protumor inflammatory milieu of the irradiated microenvironment. The prognostic biomarkers reported here may also be a potential indication for intensification of treatment. For instance, patients who achieve a complete pathologic response after neoadjuvant chemotherapy but demonstrate high-risk inflammatory biomarkers after adjuvant RT may be candidates for adjuvant chemotherapy or chemoimmunotherapy strategies.

The timing and frequency of biomarker measurements were nonprotocolized and not uniform within our study. Although we allowed each biomarker to vary over time in the regression models, we did not find an optimized time interval for biomarker measurement. Based on this multi-institutional experience, we recommend CBC with differential before RT, at the conclusion of RT, 1 month after RT, and every 3 months after RT for at least 1 year posttreatment.

Other limitations of this study include its retrospective nature and sample size. Although our study represents the patient population of 2 tertiary care centers, our results may not be extrapolatable to all practice settings. Our cohort was nonrandomized and included both stage II and stage III TNBC. Although stage and other predictors were included in multivariable analysis, our sample was underpowered, and we could not fit a model that included all relevant predictors. Given selection bias and nonrandomization, patients who received mastectomy or neoadjuvant chemotherapy were likely at greater baseline risk for recurrence and mortality. Therefore, it is unlikely that the receipt of neoadjuvant chemotherapy or mastectomy causally worsened survival, which would conflict with evidence from large randomized trials, but rather that the correlation observed with these 2 variables relates to the imbalance between groups owing to selection bias.[37,38] Although this imbalance is a notable limitation, our multivariable analysis affirms that NLR provides independent, unique, and significant prognostic information irrespective of whether patients received neoadjuvant chemotherapy or mastectomy.

A further limitation of our study is the correlational, rather than causational, relationships of biomarkers and outcome. Preradiation markers were not analyzed in light of the significant variability in time of collection in relation to RT, surgery, and chemotherapy, as well as a large number of patients with missing pre-RT markers, although the prognostic meaning of a marker measurement collected at only 1 time point before RT is debatable. This is because the pattern of markers over time is more likely to inform the underlying disease pathophysiology and probability of recurrence and is why we chose to incorporate time-varying markers in our multivariable analysis. Furthermore, because most of our patients were treated with conventionally fractionated RT, we could not establish a dose-response relationship between radiation dose and biomarker quantification. The dose-response relationship between RT and inflammatory biomarkers should be examined in conventional and hypofractionated regimens as well as dosimetric studies of nodal, cardiopulmonary, and marrow irradiation. An additional limitation to this study is the heterogeneity of chemotherapy regimens and variance of chemotherapy sequence with RT. Our study did not find any relationship between neoadjuvant or adjuvant chemotherapy sequence and inflammatory biomarkers. However, future studies should confirm this finding with a well-powered prospective study.

Conclusions

Radiation-induced elevations in NLR may be an independent prognostic biomarker for LRF, PFS, and OS in patients with stage II-III TNBC. Preclinical hypotheses purporting mechanisms of tumor recurrence through increases in neutrophil and macrophage activity with concurrent immunosuppression in an irradiated microenvironment appear to be supported by the clinical findings reported here. The inflammatory biomarkers used in this study are routinely obtained, cost-effective, easily accessible, and readily calculated from conventional blood tests. Future clinical trials may benefit from incorporating systemic inflammatory biomarkers in studying TNBC outcome and evaluating methods of attenuating systemic inflammation in patients with poor prognostic inflammatory biomarkers after radiation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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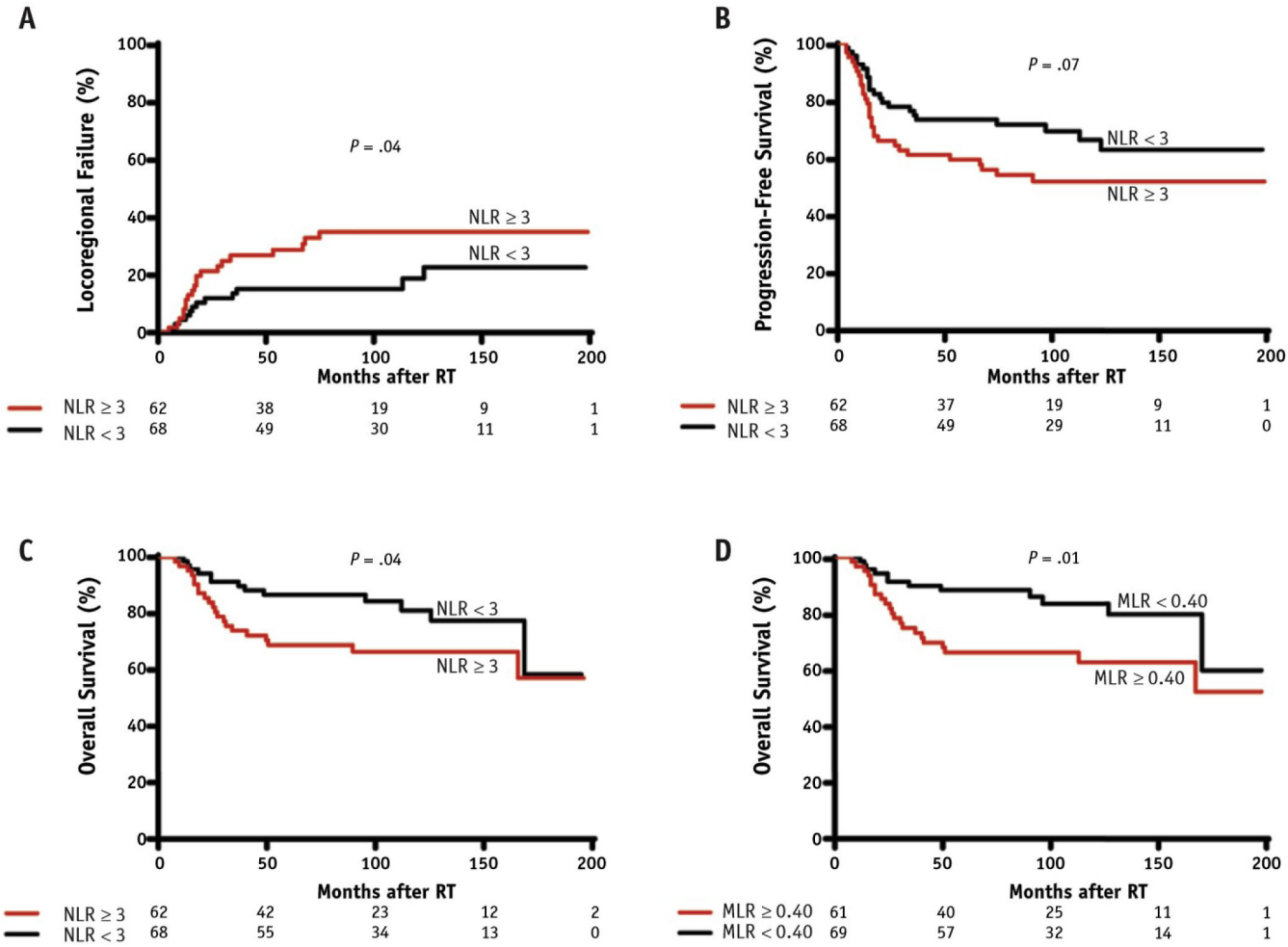

Fig. 1.

High neutrophil:lymphocyte ratio (NLR) after radiation predicts poor outcome in triple-negative breast cancer. At-risk subjects are indicated along the x-axis. (A) Competing risk analysis of locoregional failure (LRF) comparing patients with NLR <3 and NLR ≥3. Kaplan-Meier curves for the outcome of (B) progression-free survival (PFS) comparing patients with NLR <3 and NLR ≥3, (C) overall survival (OS) comparing patients with NLR <3 and NLR ≥3, and (D) OS comparing patients with monocyte:lymphocyte ratio (MLR) <0.40 and MLR ≥0.40.

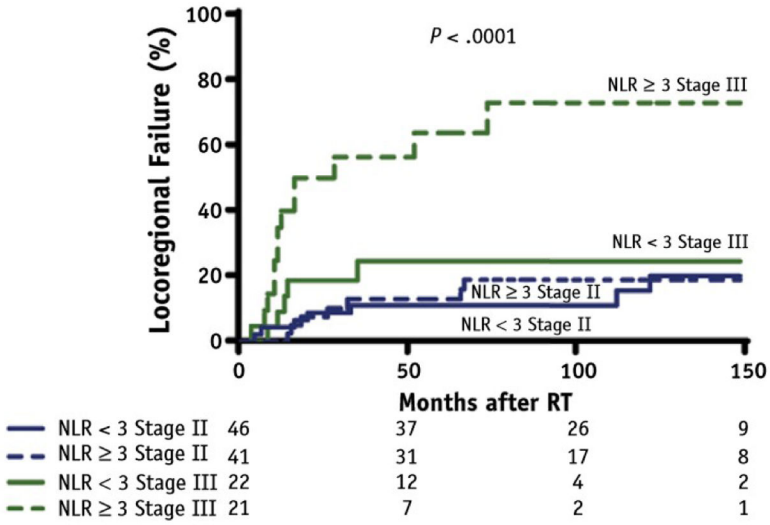

Fig. 2.

Competing risks analysis of locoregional failure (LRF) with stage II versus III and neutrophil:lymphocyte ratio (NLR) <3 versus NLR ≥3.

Table 1

Clinicopathologic characteristics

Parameter	All patients (n = 130)
Age, mean (95% CI), y	50 (48-52)
Race, n (%)	
NH white	82 (63)
NH black	25 (19)
NH Asian	16 (12)
Other	7 (5)
Menopausal status, n (%)	
Premenopausal	62 (49)
Postmenopausal	65 (51)
Histopathology	
Invasive mammary carcinoma, no special type	120 (94)
Combined histologic grade, n (%)	
Low	1 (1)
Intermediate	23 (18)
High	101 (81)
Stage, n (%)	
II	87 (67)
III	43 (33)
Surgery, n (%)	
Lumpectomy	76 (59)
Mastectomy	53 (41)
Lymphovascular invasion at surgery, n (%)	36 (50)
Any form of systemic therapy, n (%)	126 (97)
Neoadjuvant chemotherapy, n (%)	68 (51)
Adjuvant chemotherapy, n (%)	93 (72)
Both neoadjuvant and adjuvant chemotherapy, n (%)	33 (25)
RT target region, n (%)	
Whole breast or chest wall	33 (27)
With nodal coverage	89 (73)
RT tumor bed dose, median (IQR), Gy	60.4 (60.0-60.8)

Abbreviations: CI = confidence interval; IQR = interquartile range; NH = non-Hispanic; RT = radiation therapy.

Table 2

Single-predictor time-dependent competing risk analysis for locoregional failure and time-dependent Cox proportional hazards regression for progression-free survival and overall survival

	LRF			PFS			OS		
Predictor	**HR**	**95% CI**	***P* value**	**HR**	**95% CI**	***P* value**	**HR**	**95% CI**	***P* value**
ALC	0.40*	0.18-0.86	.02	0.52*	0.29-0.93	.03	0.53	0.26-1.1	.07
PLR	1.0	1.0-1.0	.1	1.0	0.99-1.0	.4	1.0	0.99-1.0	.4
MLR	2.8	0.86-9.1	.09	2.1	0.75-5.9	.2	3.8*	1.2-11	.02
NLR	1.4*	1.1-1.7	.003	1.3*	1.1-1.6	.001	1.2*	1.1-1.4	.005

Abbreviations: ALC = absolute lymphocyte count; CI = confidence interval; HR = hazard ratio; LRF = locoregional failure; MLR = monocyte: lymphocyte ratio; NLR = neutrophil:lymphocyte ratio; OS = overall survival; PFS = progression-free survival; PLR = platelet:lymphocyte ratio.

All time-dependent predictors were evaluated as continuous variables.

*Statistically significant hazard ratios.

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Table 3

Multivariable time-dependent competing risk analysis for locoregional failure and multivariable time-dependent Cox proportional hazards regression analysis for progression-free survival and overall survival

	LRF			PFS			OS		
Predictor	**HR**	**95% CI**	***P* value**	**HR**	**95% CI**	***P* value**	**HR**	**95% CI**	***P* value**
Stage II vs III	5.5*	2.6-11	<.0001	4.6*	2.6-8.3	<.0001	3.6*	1.8-7.1	.0003
NLR	1.4*	1.1-1.7	.002	1.3*	1.1-1.5	.002	1.2*	1.1-1.4	.006
Mastectomy vs lumpectomy	1.3	0.63-2.8	.5	1.9*	1.1-3.5	.03	2.4*	1.2-4.7	.02
Stage II vs III	4.7*	2.2-9.9	<.0001	4.5*	2.5-8.2	<.0001	4.3*	2.1-8.9	<.0001
NLR	1.3*	1.1-1.6	.005	1.3*	1.1-1.6	.001	1.246*	1.1-1.4	.002
Adjuvant chemotherapy	0.36*	0.17-0.79	.01	0.52*	0.27-0.98	.04	0.52	0.25-1.1	.08
Stage II vs III	5.5*	2.7-11	<.0001	4.8*	2.7-8.6	<.0001	4.5*	2.2-9.1	<.0001
NLR	1.4*	1.1-1.7	.0009	1.3*	1.1-1.6	.0004	1.3*	1.1-1.4	.0007
Neoadjuvant chemotherapy	2.5*	1.2-5.4	.02	2.018*	1.1-3.7	.02	2.316*	1.1-4.9	.03

Abbreviations: CI = confidence interval; HR = hazard ratio; LRF = locoregional failure; NLR = neutrophil:lymphocyte ratio; OS = overall survival; PFS = progression-free survival.

NLR was evaluated as a continuous variable.

*Statistically significant hazard ratios.