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CLINICAL TRIAL

Neutrophil-to-lymphocyte ratio as a predictor of survival in patients with triple-negative breast cancer

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Abstract

Purpose Peripheral blood lymphopenia and elevated neutrophil-to-lymphocyte ratio (NLR) have been associated with poor outcomes in various malignancies. However, existing literature has largely focused on baseline parameters. The aim of this study is to assess the impact of radiation therapy (RT) and chemotherapy on absolute lymphocyte counts (ALC) and NLR in relation to survival outcomes in patients with triple-negative breast cancer (TNBC).

Methods A retrospective analysis was performed on 126 patients with TNBC treated at Washington University between 2005 and 2010. Cox proportional hazard model with time-varying covariates was applied to estimate the effect of time-varying ALC and NLR separately on overall survival (OS) and disease-free survival (DFS).

Results All patients received RT and 112 patients received either neoadjuvant chemotherapy or adjuvant chemotherapy, or both. Patients deceased had lower ALC and higher NLR compared to patients alive throughout the treatment course, even 1 year after treatment completion (ALC, 1 vs. 1.3, $P=0.03$ and NLR, 3.9 vs. 2.6, $P=0.03$). High ALC was associated with superior OS on both continuous and binary scales (cutoff of 1 K/ul) (HR 0.14; 95% CI 0.05–0.34; $P<0.001$ and HR 0.28; 95% CI 0.13–0.61; $P=0.01$, respectively). Additionally, high NLR was weakly associated with inferior OS on continuous scales (HR 1.1; 95% CI 1.06–1.15; $P<0.001$).

Conclusions Post-treatment lymphopenia and NLR elevation can persist until 1 year after treatment completion. Both portend shorter survival for patients with TNBC. Our data support the use of ALC and NLR to identify high risk patients who may benefit from clinical trials rather than standard of care therapy.

Keywords Neutrophil-to-lymphocyte ratio (NLR) · Triple-negative breast cancer (TNBC) · Overall survival (OS)

Abbreviations

ALC	Absolute lymphocyte count
ANC	Absolute neutrophil count
CI	Confidence interval
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
DFS	Disease-free survival

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dNLR	Derived neutrophil-to-lymphocyte ratio
EPR	Electronic patient record
ER	Estrogen receptor
HER2	Human epidermal growth factor receptor 2
HR	Hazard ratio
MBC	Metastatic breast cancer
NK cell	Natural killer cell
NLR	Neutrophil-to-lymphocyte ratio
OS	Overall survival
PD-1	Programmed cell death protein 1
PD-L1	Programmed death ligand 1
PR	Progesterone receptor
RT	Radiation therapy
TNBC	Triple-negative breast cancer
TRL	Treatment related lymphopenia
WBC	White blood count
IL-7	Interleukin 7
IL-15	Interleukin 15

Introduction

Breast cancer is the most common malignancy and the second leading cause of cancer-related death in women. In the U.S. alone, approximately 250,000 patients are diagnosed with breast cancer each year and over 40,000 die from the disease [1]. Fortunately, the majority of patients are successfully treated, with a 5-year survival rate of around 90% for the overall population, as a result of advances in early diagnoses and improved treatment options [1]. However, 15–20% of patients are diagnosed with triple-negative breast cancer (TNBC), which is often associated with an aggressive clinical course and a high risk of early relapse [2, 3]. An overall survival (OS) of less than 30% in 5 years in these patients is observed despite initial curative local and systemic treatments [2, 4] The lack of established molecular targets, such as ER and PR hormone receptors and human epidermal growth factor receptor 2 (HER2) in TNBC renders chemotherapy the primary systemic therapy, but resistance is common [5]. Although TNBC makes up a minority of breast cancer cases, it accounts for a disproportionately high number of early relapse and deaths. There is an unmet clinical need to develop effective treatments to improve the outcome of TNBC. Identifying prognostic biomarkers as potential therapeutic targets is of great interest [2, 5].

Standard of care adjuvant therapy for patients with TNBC often includes chemotherapy and radiation, both of which are known to induce lymphopenia. Pre-treatment and treatment-related lymphopenia (TRL) has been shown to correlate with poor outcomes in numerous malignancies, including cancers of the lung, colon, pancreas, and breast along with sarcomas and glioblastomas [6–11]. In some cases, the induced lymphopenia is sustained, often lasting over 1 year, long after treatment has been delivered [6]. Given the growing body of research into better understanding the molecular pathophysiology of TNBC, efforts have been made to better predict prognosis and treatment-related outcomes. Accordingly, developing well-validated and easily derived biomarkers has proven fruitful in TNBC. For example, in the pre-treatment setting, the derived neutrophil-to-lymphocyte ratio (dNLR) and NLR may help predict survival in TNBC [12]. Therefore, this retrospective study was performed to evaluate the levels of lymphocytes and neutrophils after radiation or chemo-radiation and the prognostic role of NLR in patients with TNBC.

Patients and methods

Study population

This study was reviewed and approved by the Institutional Review Board of Washington University in St. Louis, School of Medicine. Five hundred and five patients with TNBC were retrospectively identified using outpatient and inpatient databases at Washington University in St. Louis and Barnes-Jewish Hospital between 2005 and 2010. The following eligibility criteria were used to select the study population: (1) absence of hormone or HER2/neu receptors by ASCO CAP guidelines 2013, (2) biopsy proven TNBC, (3) baseline and follow-up complete blood counts, including neutrophils and lymphocytes, performed at Barnes-Jewish Hospital and thus accessible through the electronic patient record (EPR), and (4) treatment with at least radiation therapy in the adjuvant setting, with or without neoadjuvant and/or adjuvant chemotherapy. Patients were excluded if baseline CBC data were not in the EPR, if they got chemotherapy or radiation therapy before the baseline data, or if they received treatment at a different institution. Most initially screened patients did not meet the inclusion criteria.

Data collection

Information related to known prognostic factors in TNBC was obtained from the medical records of each patient. Collected information included type of surgical procedure, if performed, along with date of birth with current age, date of diagnosis, pathological response after neoadjuvant chemotherapy (if applicable), radiation dose, and finally the exact dates for the beginning and the end of treatment for neoadjuvant chemotherapy, adjuvant chemotherapy, and adjuvant radiation therapy. None of the patients received radiation therapy in the neoadjuvant setting. Additionally, all patients had exact dates for lab data, date of the last follow-up as well as the cause of death and date of death (when applicable). Lab data included white blood cell count (WBC), ANC, and

ALC. NLR was calculated by dividing the number of neutrophils by the number of lymphocytes (ANC/ALC) from the complete blood count.

Statistical analysis

Patient baseline characteristics were summarized using descriptive statistics. Associations between two categorical characteristics were examined by Pearson's Chi-square test. Wilcoxon rank sum test was used to compare quantitative variables including ALC and NLR at each time point between patients alive and deceased. Raw unadjusted *P*-value and false discover rate (FDR) adjusted *P*-value (for multiple testing correction) were reported. For other quantitative variables, two-sample *T*-test was performed to derive the *P*-value. OS was defined as the time interval from date of diagnosis to date of death or latest follow-up visit, whichever came first. DFS was defined as the time interval from the date of diagnosis to either date of relapse, date of death, or date of latest follow-up visit, whichever came earlier. For baseline NLR and ALC, the Kaplan–Meier product limit estimator method was used to estimate OS and DFS probability, after which point K-M curves were generated. Log-rank test was used to compare OS and DFS differences between patient groups. The Cox proportional hazard model was used to estimate Hazard ratio (HR) with 95% confidence interval (CI). For time-varying NLR and ALC, all measurements across times points from baseline to last follow up were used. The time-varying Cox model was applied to estimate the effect of the time-varying variable on survival endpoints with HR (95% CI) and Wald test *P*-value reported. The classical time-varying Cox proportional hazard model was applied to investigate the effect of time-varying ALC and NLR on patient survival. Briefly, the longitudinal ALC and NLR measurements and the dates of the measurements of a patient over time were broken into multiple consecutive intervals of time. Each time interval was indicated by start and end times, accompanied with the measurement of ALC and NLR at the start of the time interval, and an event indicator recording whether or not an event of interest occurred during that time interval. If an event occurred during the time interval, then the end time was the date of the event. The multivariate time-varying Cox model was further fitted by incorporating other covariates including age, race, menopausal status, clinical stage, and neoadjuvant chemo to estimate the adjusted HR of the time-varying variable. NLR and ALC were analyzed for both continuous scale and binary scale with NLR dichotomized by 3 and ALC by 1. The likelihood ratio test was applied to derive the overall significance for variables with 3 or more categories in the Cox model. The concordance index was calculated with standard error (SE) to indicate the overall discriminative ability of a multivariate Cox model. We conducted a sensitivity examination by performing the time-varying survival analyses of ALC and NLR after excluding the three time points with relatively few sample size, namely post neoadjuvant chemotherapy, prior to adjuvant chemotherapy, and post adjuvant chemotherapy.

Results

Baseline characteristics of patients

126 patients met the required eligibility criteria, including 95 alive and 31 deceased. All deaths were attributable to breast cancer. Among all patients, 17 had recurrent disease after initial treatment, and 12 died from disease progression. Median follow-up was 6.6 years. Baseline demographic information on these patients is provided in Table 1. The median age of the patients was 55.4 (50.5–63.6) years. All patients were female. 59.5% were Caucasian ($N=75$) and 40.5% were African American ($N=51$). The majority of patients had early stage disease, including 34.1% with stage I ($N=43$) and 50.0% with stage II ($N=63$) disease, while 15.9% had advanced stage III ($N=20$). All patients received radiation therapy. 88.9% received chemotherapy ($N=112$), either in the neoadjuvant or adjuvant setting, or both. Among them, 31.7% received neoadjuvant chemotherapy only ($N=40$); 52.4% received adjuvant chemotherapy only ($N=66$); 4.8% received chemotherapy in both settings ($N=6$). 14 patients did not get any form of chemotherapy. Median age, race, menopausal status, median baseline ALC, and NLR were fairly balanced between the alive and deceased groups.

ALC and NLR over time

A series of ALC and ANC at different treatment time points were collected. NLR was generated as the ANC/ALC ratio at each time point. We reported *P*-value as FDR *P*-value, which was calculated from Wilcoxon rank sum test comparing between deceased and alive patients at each of the 8 time points for NLR and ALC, respectively. Median baseline NLR was 2.4 for both alive and deceased group (FDR $P=0.86$). Median baseline ALC was 1.9 K/ul for patients alive and 2.0 for patients deceased (FDR $P=0.77$). Time series plots were generated to show the trend of ALC and NLR at 8 different treatment points, including baseline, post neoadjuvant chemotherapy, prior to adjuvant chemotherapy, post adjuvant chemotherapy, prior to RT, at the end of RT, 2 months after RT, and 1 year after RT. NLR was significantly higher in patients deceased compared to those alive at 1 year follow-up after RT (FDR $P=0.03$) (Fig. 1) (Tables S1, S2). Patients who received neoadjuvant chemotherapy tended to have higher NLR at the following time points:

Table 1 Baseline characteristics of all patients

	All ($N=126$)	Alive ($N=95$)	Deceased ($N=31$)	P-value
Demographic data				
Age: median (IQR) years	55.4 (50.5–63.6)	56.0 (50.5–63.6)	54.5 (43.4–60.2)	0.29
Race				0.85
Caucasian: no. (%)	75 (59.5)	57 (60)	18 (58.1)	
African American: no. (%)	51 (40.5)	38 (40)	13 (41.9)	
Menopausal status				0.77
Pre-menopausal: no. (%)	38 (30.2)	28 (29.5)	10 (32.3)	
Post-menopausal: no. (%)	88 (69.8)	67 (70.5)	21 (67.7)	
Baseline laboratory data				
ALC (K/ul): median (IQR)	1.9 (1.5–2.3)	1.9 (1.5–2.2)	2 (1.5–2.4)	0.72
NLR: median (IQR)	2.4 (1.6–3.5)	2.4 (1.6–3.4)	2.4 (1.7–3.8)	0.70
Tumor staging data				0.001
Clinical stage I	43 (34.1)	38 (40)	5 (16.1)	
Clinical stage II	63 (50)	48 (50.5)	15 (48.4)	
Clinical stage III	20 (15.9)	9 (9.5)	11 (35.5)	
Treatment data				
Radiation dose (Gy): median (IQR)	6000 (5000–6000)	6000 (5020–6000)	6000 (5000–6040)	0.36
Chemotherapy: no. (%)	112 (88.9)	85 (89.5)	27 (87.1)	0.71
Neoadjuvant chemo: no. (%)	46 (36.5)	31 (32.6)	15 (48.4)	
Adjuvant chemo: no. (%)	72 (57.1)	58 (61.1)	14 (45.2)	

IQR interquartile range, *no* number, *chemo* chemotherapy

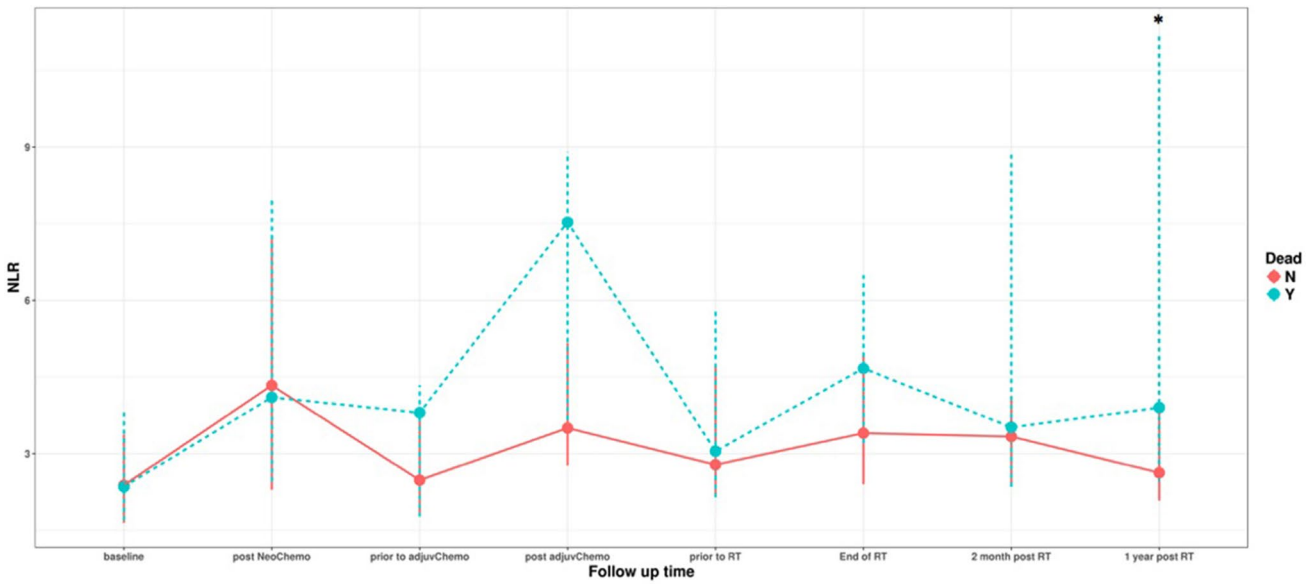

Fig. 1 Comparison of median with interquartile range (IQR) of neutrophil-to-lymphocyte ratio (NLR) in patients alive and deceased at different treatment time points, including baseline, post neoadjuvant chemotherapy, prior to adjuvant chemotherapy, post adjuvant chemotherapy, prior to radiation therapy (RT), end of RT, 2 months after RT, and 1 year after RT. Asterisk statistically significant difference between patients alive and deceased

prior to adjuvant chemotherapy, post adjuvant chemotherapy, end of RT, and 1 year after RT (Fig. 2). NLR for patients who received adjuvant chemotherapy reached its highest level right after adjuvant chemotherapy and tended to remain high at the end of RT and 2 months after RT (Fig. 2). There were significant differences in ALC between patients alive

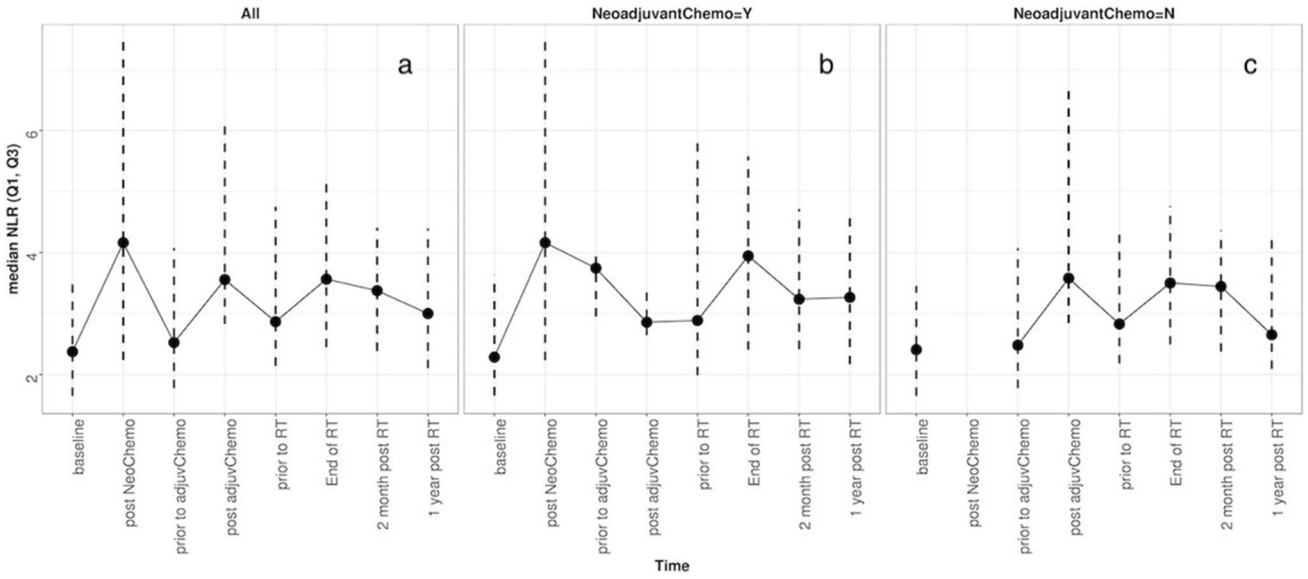

Fig. 2 Median with interquartile range (IQR) of neutrophil-to-lymphocyte ratio (NLR) in patients received neoadjuvant chemotherapy or those who did not receive neoadjuvant chemotherapy at different time points, including baseline, post neoadjuvant chemotherapy, prior to and post adjuvant chemotherapy, prior to radiation therapy (RT), end of RT, 2 months after RT, and 1 year after RT. **a** Median with interquartile range (IQR) of neutrophil-to-lymphocyte ratio (NLR) in all patients at different treatment time points, including baseline, post neoadjuvant chemotherapy, prior to and post adjuvant chemotherapy, prior to radiation therapy (RT), end of RT, 2 months after RT, and 1 year after RT. **b** Median with interquartile range (IQR) of neutrophil-to-lymphocyte ratio (NLR) in patients who received neoadjuvant chemotherapy at different treatment time points, including baseline, post neoadjuvant chemotherapy, prior to and post adjuvant chemotherapy, prior to radiation therapy (RT), end of RT, 2 months after RT, and 1 year after RT. **c** Median with interquartile range (IQR) of neutrophil-to-lymphocyte ratio (NLR) in patients who did not receive neoadjuvant chemotherapy at different treatment time points, including baseline, post neoadjuvant chemotherapy, prior to and post adjuvant chemotherapy, prior to radiation therapy (RT), end of RT, 2 months after RT, and 1 year after RT

and deceased at the end of RT (FDR $P=0.04$), 2 months after RT (FDR $P=0.03$), 1 year after RT (FDR $P=0.03$) (Fig. 3) (Tables S3, S4). Patients who received neoadjuvant chemotherapy, compared to those who did not, tended to have lower ALC until 1 year after completion of RT (Fig. 4). Greater variability in NLR and ALC was observed in deceased patients while the surviving patients exhibited a relatively stable NLR profile longitudinally. Among the patients deceased, a steady increase in NLR over baseline measurement was observed in subsequent time points. The ALC variability in the deceased patient group was significantly higher at the end of the RT. Summary statistics of ALC and NLR at different treatment points are shown in Tables S1–S4 in the Supplementary Appendix.

Median overall survival and disease-free survival by Kaplan–Meier Analysis

Binary NLR and ALC variables were created using cutoff points of 3.0 and 1.0, respectively. The use of NLR 3.0 and ALC 1.0 as the cutoff points to compare survival was based on previous studies [10, 12–23] The median OS for patients with baseline NLR ≥ 3 was 10.2 years, compared with not reached for patients with baseline NLR < 3 (HR 1.67; 95% CI 0.81–3.45; $P=0.23$) (Fig. S1). The median DFS was not reached for patients with baseline NLR ≥ 3 and baseline NLR < 3 (HR 1.1; 95% CI 0.39–3.14; $P=0.77$) (Fig. S2). The median OS for patients with baseline ALC ≥ 1 was not reached, compared with 11.3 for patients with baseline ALC < 1 (HR 0.55; 95% CI 0.19–1.57; $P=0.86$) (Fig. S3). The median DFS was not reached for patients with baseline ALC ≥ 1 and those with baseline ALC < 1; however, patients with ALC ≥ 1 appeared to have longer but not statistically significant DFS than those with ALC < 1 (HR 0.38; 95% 0.11–1.35; $P=0.82$) (Fig. S4). Earlier clinical stage was significantly associated with better OS ($P<0.001$) (Fig. S5).

NLR, ALC, and their associations with survival by time-varying Cox regression model

Data at eight treatment time points were included in the analysis, including baseline, post neoadjuvant chemotherapy, before adjuvant chemotherapy, post adjuvant chemotherapy, before RT, end of RT, 2 months after RT, and 1 year after RT. A recent meta-analysis demonstrated high NLR is associated with worse OS and DFS in breast cancer patients and suggested that NLR is a good prognostic marker for breast cancer survival [10].

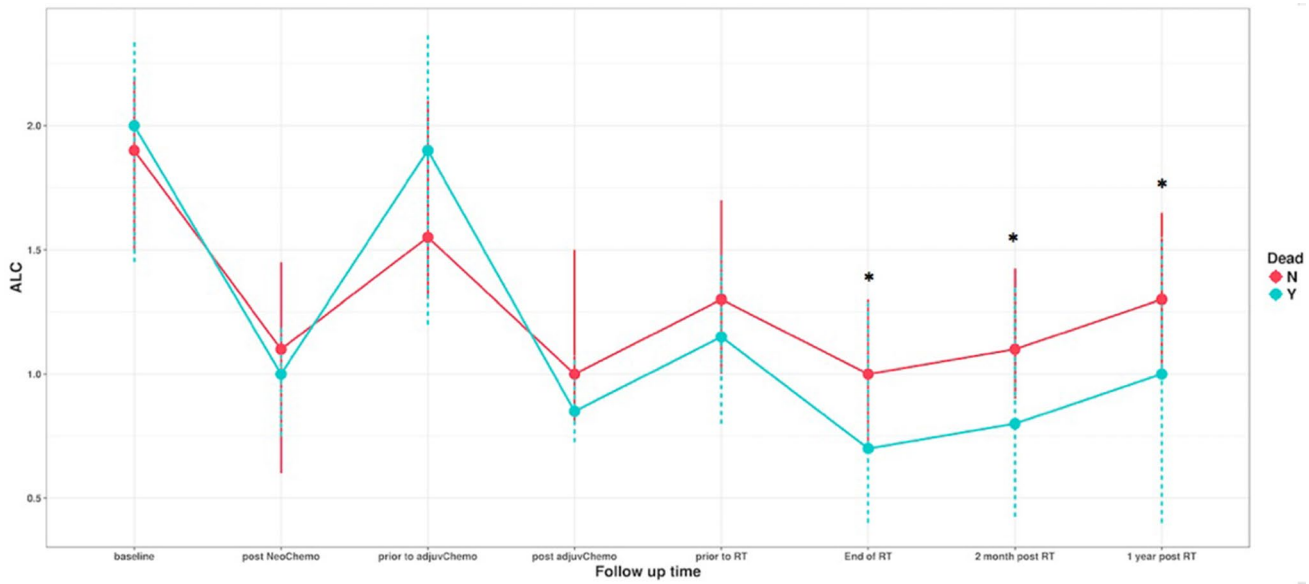

Fig. 3 Comparison of median with interquartile range (IQR) for absolute lymphocyte count (ALC) in patients alive and deceased at different treatment time points, including baseline, post neoadjuvant chemotherapy, prior to adjuvant chemotherapy, post adjuvant chemotherapy, prior to radiation therapy (RT), end of RT, 2 months after RT, and 1 year after RT. Asterisk statistically significant difference between patients alive and deceased

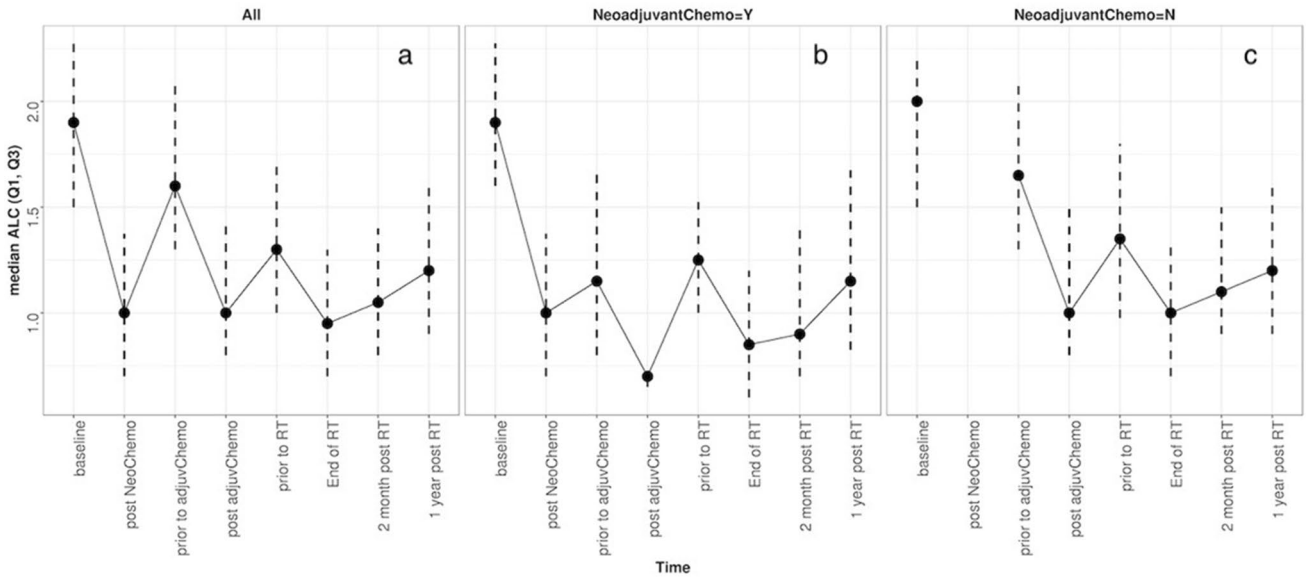

Fig. 4 Median with interquartile range (IQR) of absolute lymphocyte count (ALC) in patients who received neoadjuvant chemotherapy at different treatment time points, including baseline, post neoadjuvant chemotherapy, prior to and post adjuvant chemotherapy, prior to radiation therapy (RT), end of RT, 2 months after RT, and 1 year after RT. **a** Median with interquartile range (IQR) of absolute lymphocyte count (ALC) in all patients at different treatment time points, including baseline, post neoadjuvant chemotherapy, prior to and post adjuvant chemotherapy, prior to radiation therapy (RT), end of RT, 2 months after RT, and 1 year after RT. **b** Median with interquartile range (IQR) of absolute lymphocyte count (ALC) in patients who received neoadjuvant chemotherapy at different treatment time points, including baseline, post neoadjuvant chemotherapy, prior to and post adjuvant chemotherapy, prior to radiation therapy (RT), end of RT, 2 months after RT, and 1 year after RT. **c** Median with interquartile range (IQR) of absolute lymphocyte count (ALC) in patients who did not receive neoadjuvant chemotherapy at different treatment time points, including baseline, post neoadjuvant chemotherapy, prior to and post adjuvant chemotherapy, prior to radiation therapy (RT), end of RT, 2 months after RT, and 1 year after RT

We performed multivariate Cox proportional-hazards models with time-varying NLR or ALC analysis adjusted for age, race, menopausal status, clinical stage, and chemotherapy status. The time-varying NLR multivariate Cox model demonstrated that high NLR was associated with inferior OS on continuous scales (HR 1.1; 95% CI 1.06–1.15; $P < 0.001$) (Table 2). The multivariate Cox model with time-varying ALC analysis showed that high ALC was associated with superior OS on continuous scales (HR 0.14; 95% CI 0.05–0.34; $P < 0.001$) (Table 3). Both models suggested that advanced stage (stage III compared to stage I) was consistently associated with worse OS (HR 9.51; 95% CI 2.54–35.62; $P < 0.001$ and HR 13.11; 95% CI 2.1–81.76; $P < 0.01$ and HR 7.51; 95% CI 1.93–26.51; $P < 0.01$, respectively), as well as worse DFS (HR 13.11; 95% CI 2.1–81.76; $P < 0.01$ and HR 13.11; 95% CI 2.1–81.76; $P < 0.01$). In addition, adjuvant chemotherapy was associated with better DFS in both models on continuous scales (HR 11.6; 95% CI 1.99–67.64; $P < 0.01$ and HR 10.38; 95% CI 1.74–62.13; $P = 0.01$, respectively) (Tables 2, 3). Multivariate Cox model with time-varying NLR and ALC analysis on binary scale suggested similar results (Tables S5, S6).

Discussion

In this retrospective study, we analyzed the effect of radiation and chemotherapy on ALC and NLR in relation to survival outcomes of patients with early stage TNBC. We demonstrated that the median ALCs were in the normal range, without any significant differences, prior to initiation of therapy in patients deceased or alive. Upon completion of radiation, lymphocyte counts declined in both groups as expected. Lymphopenia persisted for months and up to a 1 year after RT in patients deceased, whereas lymphocyte counts gradually recovered during follow-up, with median ALC improving back to the level prior to RT by 1 year after RT in patients alive. In addition, ALC was significantly

Table 2 Multivariate Cox proportional hazards regression model with time-varying NLR analysis on continuous scale

Variable	OS concordance/ SE = 0.7680/0.0592		DFS concordance/ SE = 0.7973/0.0815	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
NLR (continuous)	1.1 (1.06–1.15)	< 0.001	1.06 (0.99–1.13)	0.07
Race (caucasian vs. AA)	1.47 (0.62–3.46)	0.38	1.38 (0.41–4.58)	0.60
Age (continuous)	0.97 (0.93–1.01)	0.18	0.96 (0.9–1.03)	0.25
Stage		0.0032		0.02
Stage (II vs. I)	2.91 (0.97–8.74)	0.06	2.96 (0.81–10.81)	0.10
Stage (III vs. I)	9.51 (2.54–35.62)	< 0.001	13.11 (2.1–81.76)	< 0.01
Menopausal status (post vs. pre)	2.55 (0.68–9.51)	0.16	1.17 (0.19–7.26)	0.87
Neoadjuvant chemo (yes vs. no)	0.72 (0.22–2.36)	0.59	2.83 (0.69–11.58)	0.15
Adjuvant chemo (yes vs. no)	0.87 (0.27–2.86)	0.82	11.6 (1.99–67.64)	< 0.01

NLR neutrophil-to-lymphocyte count ratio, *OS* overall survival, *DFS* disease-free survival, *SE* standard error, *HR* hazard ratio, *Chemo* chemotherapy

Table 3 Multivariate Cox proportional hazards regression model with time-varying ALC analysis on continuous scale

Variable	OS Concordance/ SE = 0.8059/0.0592		DFS Concordance/ SE = 0.7791/0.0815	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
ALC (continuous)	0.14 (0.05–0.34)	< 0.001	0.94 (0.42–2.08)	0.88
Race (caucasian vs. AA)	1.33 (0.59–2.97)	0.49	1.47 (0.45–4.84)	0.53
Age (continuous)	0.95 (0.91–1)	0.04	0.97 (0.9–1.04)	0.33
Stage		0.0105		0.0328
Stage (II vs. I)	2.56 (0.84–7.84)	0.10	2.95 (0.81–10.71)	0.10
Stage (III vs. I)	7.51 (1.93–26.51)	< 0.01	13.11 (2.1–81.76)	< 0.01
Menopausal status (post vs. pre)	4.7 (1.24–17.79)	0.22	1.03 (0.16–6.59)	0.98
Neoadjuvant chemo (yes vs. no)	1 (0.36–2.8)	0.99	2.78 (0.67–11.55)	0.16
Adjuvant chemo (yes vs. no)	0.8 (0.28–2.34)	0.69	10.38 (1.74–62.13)	0.01

ALC absolute lymphocyte count, *OS* overall survival, *DFS* disease-free survival, *SE* standard error, *HR* hazard ratio, *chemo* chemotherapy

lower in patients deceased compared to those alive at the end of RT, 2-month, and 1-year follow-up after RT. Median NLR was consistently higher in patients deceased than those alive after neoadjuvant chemotherapy, and the difference was statistically significant at 1 year after completion of treatment. NLR < 3 and ALC ≥ 1 are both associated with non-significant better OS.

We collected ALC and NLR at 8 time points throughout the treatment and follow-up course. Given the fact that some patients only received neoadjuvant chemotherapy or adjuvant chemotherapy, data at the associated three time points, namely post neoadjuvant chemotherapy, prior to adjuvant chemotherapy, post adjuvant chemotherapy, were not available for all the patients. To examine the sensitivity of the results to these three time points with relatively few patients, we further performed a Multivariate Cox Proportional Hazards Regression model with time-varying analysis after excluding those the three time points. The results were overall similar to the analysis using all the time points, indicating that NLR and ALC are still prognostic of overall survival (Tables S7–S10).

While the exact mechanism underlying TRL is not entirely understood, it is possible that lymphopenia adversely influences immune surveillance and destruction of micrometastatic malignant cells, leading to recurrence. Previous studies have shown that patients with intense tumor infiltrating lymphocyte infiltration (TILs) tend to have longer survival times than those who do not [6, 24]. Several studies also suggest that effective engagement of the immune system, demonstrated by elevated expression of B-cell and T-cell-related metagenes, can facilitate the risk reduction of distant metastasis, thereby improving survival [25–27]. The complex interplay between the innate and adaptive immune response represented by neutrophils and various types of T-cells, tumor cells, and the tumor stroma/microenvironment, may provide a possible mechanism behind these findings [12] Our study suggests that peripheral blood ALC and NLR post-therapy may serve as a surrogate of anti-cancer immune capability of the host. This is also supported by a previous study by Verma et al., which used flow-cytometry to assess circulating lymphocyte levels in breast cancer patients before and after chemotherapy, and found that levels of B, T, and natural killer (NK) cells were significantly reduced 2 weeks after chemotherapy and that B and CD4 + T cells remained significantly depleted even 9 months post chemotherapy [28].

In addition to ALC, we observed that NLR, a potential marker of systemic inflammation, pre-treatment and at the completion of RT as well as at follow up time points, was prognostic of survival outcomes. There were significant differences in NLR between patients living and deceased at the completion of RT and at 2 month and 1 year time points after RT, correlating with the significantly lower ALCs in the deceased group compared to the alive group at these time points. Of particular importance is our observation that lower NLR (< 3) was significantly associated with longer OS throughout the treatment course and subsequent follow-up.

Our data add to existing knowledge regarding a correlation between NLR and prognosis in patients with breast cancer [12, 23, 29–32]. However, the studies in breast cancer focused primarily on the pre-treatment setting. For example, Azab et al., demonstrated that patients in the highest pretreatment NLR quartile (NLR > 3.3) had higher 1-year and 5-year mortality rates compared with those in the lowest quartile (NLR < 1.8), suggesting that NLR could be an independent and significant predictor of short-term and long-term mortality in breast cancer patients [23]. Similarly, a study focusing on luminal A breast cancer found that pretreatment NLR > 2.5 was independently associated with poor prognosis [33]. A study by Mazouz et al. on patients with metastatic breast cancer (MBC) reported similar findings [34]. Our analysis represents the first study that analyzed post-therapy elevated NLR in association with survival outcomes in the TNBC subset of breast cancer patients. Similarly, although the association between pretreatment lymphopenia with poor outcomes in breast cancer patients has been consistently described [12, 31, 35–37], few focused on the post-treatment setting and TNBC subset. Our study provides additional evidence that TRL and post-treatment NLR are associated with TNBC survival.

Our data of TRL in TNBC and its association with survival outcomes are in line with observations from other cancer types. Grossman et al. reported that severe TRL at 2 months after RT was independently associated with shorter survival from tumor progression [7]. Campian et al. investigated TRL in patients with stage III non-small-cell lung cancer (NSCLC) and revealed an association between severe reductions in total lymphocyte count (TLC) and reduced survival [6]. Similar findings were recently reported in locally advanced cervical cancer [38]. Our findings are consistent with previous studies showing that lower NLR (< 3) is associated with significantly longer OS, thereby exhibiting that the beneficial effect of immune system engagement on survival persists beyond the treatment course.

In the era of immunotherapies, a functional immune system, especially with adequate lymphocyte-involvement, is critical for the success of immunotherapeutic agents. Efforts have been made to stimulate an immune system response towards specifically targeted cancer cells, namely through checkpoint inhibitors targeting cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1), and programmed death ligand 1 (PD-L1) and PD-L2. It has been postulated that adequate lymphocytes are required in order for checkpoint inhibitor therapy to be effective, necessitating the importance of further research and development of therapies to address

TRL [5]. There are several ongoing clinical trials to test these agents with or without checkpoint inhibitors in solid tumors, including TNBC [39, 40].

Thus, researchers have been focusing on approaches to mitigate or correct TRL [41]. A recent study reported that recombinant human interleukin 7 (IL-7) can raise lymphocyte counts and improve lymphocyte function in patients with sepsis [27]. Another cytokine, interleukin 15 (IL-15), was shown to improve CD8 and NK cells levels and function in patients with leukemia [42]. It remains to be seen whether these approaches can improve patient outcomes.

Our study is inherently limited, however, in being retrospective and including a relatively small number of patients from a single institution. Moreover, with few numbers of patients in each subgroup, outliers are more likely to influence outcomes, for which larger, prospectively derived studies are needed to substantiate our work by assessing the relationship between baseline ALC and NLR values and clinical outcomes, which our study was not able to fully assess. Regardless, our work adds to growing knowledge in the field regarding the importance of the immune reactivity in TNBC.

Conclusions

This is the first study, to our knowledge, investigating the effect of treatment related ALC and NLR on survival in patients with TNBC. Our study showed high NLR and low ALC was associated with inferior OS (HR 1.1; 95% CI 1.06–1.15; $P < 0.001$ and HR 0.14; 95% CI 0.05–0.34; $P < 0.001$, respectively), suggesting that treatment approaches to improve host immune parameters may be important.

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Author contributions DAP was involved in data collection and the writing, revision and approval of the manuscript. JX was involved in statistical data analysis and the writing, revision and approval of the manuscript. JL was involved in statistical data analysis and the writing, revision and approval of the manuscript. BH was involved in the writing, revision and approval of the manuscript. ST was involved in the writing, revision and approval of the manuscript. CXM was involved in study design and the writing, revision and approval of the manuscript. JLC was involved in study design, study supervision and the writing, revision and approval of the manuscript.

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Data availability All data and material are available upon request.

Compliance with ethical standards

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

Consent for publication All authors listed above have reviewed and verified the manuscript for accuracy. All authors have consented to be an author and publish the manuscript.

Ethical approval This retrospective study protocol was approved by Washington University Institutional Review Board (IRB) (Reference #: 201406126). Upon approval, Washington University agreed to follow the Declaration of Helsinki, Good Clinical Practice guidelines, and the applicable parts of the United States Code of Federal Regulations.

Human and animal rights All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

Informed consent Informed consent was not required for this retrospective analysis.

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