Published OnlineFirst March 26, 2018; DOI: 10.1158/1078-0432.CCR-17-1323

Precision Medicine and Imaging

Clinical Cancer Research

Higher Absolute Lymphocyte Counts Predict Lower Mortality from Early-Stage Triple-Negative Breast Cancer

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Abstract

Purpose: Tumor-infiltrating lymphocytes (TIL) in pretreatment biopsies are associated with improved survival in triple-negative breast cancer (TNBC). We investigated whether higher peripheral lymphocyte counts are associated with lower breast cancer–specific mortality (BCM) and overall mortality (OM) in TNBC.

Experimental Design: Data on treatments and diagnostic tests from electronic medical records of two health care systems were linked with demographic, clinical, pathologic, and mortality data from the California Cancer Registry. Multivariable regression models adjusted for age, race/ethnicity, socioeconomic status, cancer stage, grade, neoadjuvant/adjuvant chemotherapy use, radiotherapy use, and germline *BRCA1/2* mutations were used to evaluate associations between absolute lymphocyte count (ALC), BCM, and OM. For a subgroup with TIL data available, we explored the relationship between TILs and peripheral lymphocyte counts.

Results: A total of 1,463 stage I–III TNBC patients were diagnosed from 2000 to 2014; 1,113 (76%) received neoadjuvant/adjuvant chemotherapy within 1 year of diagnosis. Of 759 patients with available ALC data, 481 (63.4%) were ever lymphopenic (minimum ALC <1.0 K/μL). On multivariable analysis, higher minimum ALC, but not absolute neutrophil count, predicted lower OM [HR = 0.23; 95% confidence interval (CI), 0.16–0.35] and BCM (HR = 0.19; CI, 0.11–0.34). Five-year probability of BCM was 15% for patients who were ever lymphopenic versus 4% for those who were not. An exploratory analysis ($n = 70$) showed a significant association between TILs and higher peripheral lymphocyte counts during neoadjuvant chemotherapy.

Conclusions: Higher peripheral lymphocyte counts predicted lower mortality from early-stage, potentially curable TNBC, suggesting that immune function may enhance the effectiveness of early TNBC treatment. *Clin Cancer Res; 24(12); 2851–8.* ©*2018 AACR.*

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Prior presentation: An earlier version of this work was presented in partial form at the 2015 and 2016 American Society of Oncology Annual Meetings, Chicago, Illinois.

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doi: 10.1158/1078-0432.CCR-17-1323

Introduction

Many factors influence breast cancer patients' risk of death, including age and comorbid conditions, tumor subtype, grade and stage, and treatments received. Prior studies have shown that lymphopenia, or a low peripheral blood lymphocyte count, may also be a harbinger of worse mortality from advanced carcinomas, sarcomas, and lymphomas (1–7). An association between lymphopenia and poor mortality outcomes in breast cancer was reported in 1976 (4) and later confirmed in two studies showing an associated risk of recurrence after primary surgery and neoadjuvant therapy (5, 7). Several recent studies have demonstrated lower breast cancer–specific mortality (BCM) associated with tumor-infiltrating lymphocytes (TIL) in patients treated with neoadjuvant/adjuvant chemotherapy. This is particularly notable with triple-negative breast cancer (TNBC; refs. 8–12), the breast cancer subtype that is defined by a lack of estrogen (ER) and progesterone receptor (PR) expression and of HER2 receptor overamplification and has relatively high mortality (13).

Emerging immune checkpoint inhibitors, particularly antibodies against programmed death receptor 1 (PD-1) and its ligand (PD-L1; ref. 14–17), have shown promising results across cancer

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Translational Relevance

Tumor-infiltrating lymphocytes (TIL) in pretreatment biopsies are associated with lower mortality in triple-negative breast cancer (TNBC). We investigated whether higher peripheral lymphocyte counts are associated with lower breast cancer–specific mortality (BCM) and overall mortatlity (OM) in TNBC. On multivariable analysis, we found that higher minimum absolute lymphocyte count, but not absolute neutrophil count, predicted improved OM and BCM. Five-year probability of BCM was 15% for patients who were ever lymphopenic versus 4% for those who were not. An exploratory analysis ($n = 70$) showed a significant association between TILs and higher peripheral lymphocyte counts during neoadjuvant chemotherapy. The results contribute to an emerging understanding of immune function in TNBC. In future clinical trials for TNBC, peripheral lymphocyte counts should be explored in connection with TILs as a potential biomarker for treatment efficacy and cancer mortality.

types, including in early-phase clinical trials among TNBC patients (16–19). Given this evolving therapeutic area, we investigated whether peripheral lymphocyte levels as measured by absolute lymphocyte count (ALC) have a similar prognostic effect as TILs in TNBC (8–12). We took advantage of the Oncoshare database, which integrates electronic medical records (EMR) of two Northern California health care systems with records of the California Cancer Registry [CCR, a contributing registry of the Surveillance, Epidemiology and End Results (SEER) program; ref. 20], to evaluate the following three aims: (i) factors associated with lymphopenia; (ii) the association between ALC and mortality; and (iii) the variation in strength of association between ALC and mortality over time among patients diagnosed with early-stage TNBC from 2000 to 2014. As a secondary exploratory analysis, we evaluated the relationship between peripheral blood lymphocytes and TILs among 70 patients with pretreatment TIL information.

Materials and Methods

Oncoshare data resource

Data were collected using Oncoshare, a breast cancer outcomes research database that integrates EMR data from breast cancer patients treated at Stanford University Hospital (Stanford, CA) and/or the community-based Palo Alto Medical Foundation (20, 21). Oncoshare links patient-level clinical data from EMRs to population-based CCR (SEER) data (22–24). The CCR, with mandated statewide reporting, provides a gold standard for patient identification, cancer staging, and long-term follow-up. Methods involved in developing Oncoshare, including validation of the data linkage approach, have been published (20, 21). In brief, clinical data from the two health care systems were extracted from EMRs, and drug data elements and laboratory results from EMRs were mapped to a standardized lexicon (25). CCR records were requested for all patients with breast cancer treated at either of the two health care systems. Data from EMRs and CCR were then linked on an individual patient level and deidentified before research use. All research was approved by the Institutional Review Boards of Stanford University, Palo Alto Medical Foundation Research Institute, and the State of California.

Variable definitions and sources

Variables from the CCR included age and race/ethnicity; cancer stage, grade, ER/PR, and HER2 status; and summary of initial treatment course, including surgery, neoadjuvant/adjuvant chemotherapy, and radiotherapy, administered by any treating institution in California. TNBC was defined as breast cancer that had no ER/PR staining using IHC and no overexpression or amplification of HER2. BCM and overall mortality (OM) data were derived from the CCR, which collects this information using routine linkages with the Social Security Death Master File, the Registry of Motor Vehicles, and other national databases (26). OM was defined as the time from breast cancer diagnosis to death for any reason or the day of last follow-up. BCM was defined as the time from breast cancer diagnosis to breast cancer–specific death or the day of last follow-up. Neighborhood socioeconomic status (nSES, at the Census block-group level and in terms of statewide quintiles) was assigned on the basis of Census data using the validated Yost index (27).

EMR data included receipt of neoadjuvant/adjuvant chemotherapy and radiotherapy and laboratory values. Ever lymphopenic was defined as having an ALC <1 K/μL, and ever neutropenic as having an absolute neutrophil count (ANC) <1 K/μL, anytime after TNBC diagnosis. Minimum ALC was defined as the lowest value of all reported ALC measures anytime after TNBC diagnosis. If a patient had only one ALC value measured, then it was set as the minimum. Minimum percent lymphocyte count was included as an additional measure of lymphopenia.

Germline *BRCA1* and *BRCA2 (BRCA1/2)* mutation status [tested and positive for a deleterious mutation; tested and negative for a deleterious mutation; tested and had a variant of uncertain significance (VUS); not tested or unknown] was obtained from Myriad Genetics, Inc., which was the single source of clinical *BRCA1/2* testing during the study timeframe (28).

Inclusion and exclusion criteria

All patients in the Oncoshare database diagnosed with stages I–III TNBC between January 2000 and May 2014 were eligible. To restrict consideration of neoadjuvant/adjuvant chemotherapy to treatment, which was undertaken for curative intent rather than for metastatic cancer, the chemotherapy receipt variable was limited to within one year of the initial breast cancer diagnosis. Patients were followed through May 31, 2014.

Exploratory analysis of TILs and peripheral blood lymphocytes

In an exploratory analysis of 70 patients who participated in a phase II clinical trial of neoadjuvant carboplatin, gemcitabine, and iniparib (PrECOG 0105; refs. 12, 29), we evaluated the association between pretreatment stromal TILs and peripheral blood lymphocytes. Stromal TIL values were reported in deciles (0 = 0%, 1 = 10%, 2 = 20%, etc., of tumor stroma containing lymphocytes not in direct contact with tumor cells) as per consensus guidelines (30). Pretreatment and minimum lymphocyte values during treatment and up to 30 days posttreatment were reported as percentages and absolute counts.

Statistical analysis

A logistic regression model was fit to whether or not a patient was ever lymphopenic as a function of cancer stage, grade,

Published OnlineFirst March 26, 2018; DOI: 10.1158/1078-0432.CCR-17-1323

neoadjuvant/adjuvant chemotherapy use, radiotherapy use, nSES, race/ethnicity, *BRCA1/2* mutation status (positive for a deleterious mutation; negative for a deleterious mutation and/or having a VUS, the latter two of which were combined as a single category given their similar clinical management; untested or unknown), age at diagnosis, and ever neutropenic. ORs and 95% confidence intervals (CI) were estimated.

Multivariable Cox regression models were fit to examine the association between minimum ALC and OM, and minimum ALC and BCM, adjusting for all covariates described above. Data from patients who were alive through the last follow-up date were censored. HRs and 95% CIs were computed. To determine 5-year survival and the 95% confidence limits among ever- and never-lymphopenic patients, the survest function from the rms package in R was implemented using estimates from our fitted models and the mean and mode values for each of the continuous or categorical predictors, respectively (31). For models that adjusted for additional covariates, primary analyses used a complete case approach, excluding patients with missing data. Given the high rate of patients without germline *BRCA1/2* testing or reported ALC values, multiple imputation methods were implemented using the default settings of the MICE package in R (32), with five imputations used (33, 34). As a sensitivity analysis on using minimum ALC to measure lymphopenia, multivariable Cox regression models were fit, replacing minimum ALC with minimum percent lymphocyte count and adjusting for the covariates described above.

We used mixed effects logistic regression techniques to evaluate the association between mortality outcomes and time-varying ALC. All ALC values within each time interval were included. The two outcomes were indicators for whether or not OM or BCM occurred. The models included time since diagnosis, time-varying ALC value, and their interaction (representing how the magnitude of association between ALC and mortality varied over time). A subject-specific random effect was included to account for correlation of observations within a given patient over time. To report the effect of ALC on mortality by years since diagnosis, we calculated contrasts from the interaction term for year 1 through year 6. Because of convergence issues, no other covariates were included in these models. OM and BCM for ever-lymphopenic and never-lymphopenic patients were displayed using Kaplan–Meier curves. For the exploratory analysis of baseline TILs and peripheral lymphocyte counts, we used the Spearman rank correlation test and reported the associated P value and Spearman ρ. We performed all statistical analyses in R (Version 3.2.2). All tests were two-sided and statistical significance used an α threshold of 0.05.

Results

Patient characteristics

From January 1, 2000, through May 31, 2014, 1,463 patients were diagnosed with stages I–III TNBC and treated at Stanford University Hospital and/or Palo Alto Medical Foundation (Supplementary Fig. S1). Mean age at diagnosis was 54 years and most were non-Hispanic (NH) White (72%), followed by NH Asian/Pacific Islander (14%). Most were of the second highest nSES quintile. Nearly half (49%) had stage II and three quarters had high-grade disease (73%). Three quarters received either adjuvant or neoadjuvant chemotherapy (76%), and approximately half (46%) received adjuvant radiotherapy. Nearly one quarter (23%) were tested clinically for *BRCA1/2* mutations, of whom 80 (24% of tested patients) carried a deleterious mutation in either or both genes. Most (81%) were never neutropenic (ANC <1.0 K/μL), yet most (63.4%) were lymphopenic (ALC <1.0 K/μL) with a mean minimum ALC value of 0.9 K/μL (Table 1).

Over a median follow-up time of 4.5 years, 352 (24%) patients died, with 222 (63%) of these deaths from breast cancer. Approximately half (759, 52%) had at least one ALC value reported. In comparison with the full cohort, those with ALC values were slightly more likely to receive neoadjuvant/adjuvant chemotherapy (81% vs. 76%) and *BRCA1/2* testing (29% vs. 23%; Table 1).

Factors associated with lymphopenia

On our primary multivariable analysis, which only included variables that did not vary with time, higher stage was positively associated with lymphopenia (OR = 1.84; 95% CI, 1.03–3.28; Table 2). Exploratory analysis including variables with temporal sequences showed that neoadjuvant/adjuvant chemotherapy use (OR = 2.66; 95% CI, 1.72-4.15), higher nSES (OR = 1.18; 95% CI, 1.03–1.36), ever being neutropenic (OR = 6.05; 95% CI, 3.46–11.35), and higher stage (OR = 1.73; 95% CI, 1.02–3.01) were positively associated with lymphopenia (Supplementary Table S1).

Minimum ALC, OM, and BCM

The number of reported ALC values ranged from 1 to 117 (mean 14, median 10). No significant difference in OM or BCM was observed between patients who had <10 (the median), versus ≥10 measured ALC values, and only minimal increases in OM (HR = 1.02; 95% CI, 1.01–1.03) and BCM (HR = 1.02; 95% CI, 1.01–10,2) were associated with the number of measured ALC values when that number was analyzed as a continuous variable. On multivariable analysis, patients with higher minimum ALC had significantly lower overall mortality than those with lower minimum ALC (HR = 0.23; 95% CI, 0.16–0.35). Other predictors of lower OM were receiving neoadjuvant/adjuvant chemotherapy (HR = 0.53; 95% CI, 0.34–0.84) and higher nSES (HR = 0.85; CI, 0.74–0.97). Higher stage was associated with higher OM (III vs. I HR = 4.25; CI, 2.69–6.74; Table 3; Fig. 1).

Higher minimum ALC was also significantly associated with lower BCM (HR = 0.19; CI, 0.11–0.34), as was higher nSES (HR = 0.85; CI, 0.71–1.00). Higher stage (stage III vs. stage I HR = 7.59; CI, 3.83–15.0) was associated with higher BCM (Table 4; Fig. 1). The model predicted an absolute probability of 5-year BCM among ever-lymphopenic patients of 15%, compared with 4% for never-lymphopenic patients (95% CI, 12%–17% for ever-lymphopenic patients vs. 95% CI, 1%–6% for never-lymphopenic patients).

We observed no interaction between carriage of deleterious *BRCA1/2* mutations and minimum ALC in a complete case analysis (HR = 0.58; CI, 0.30–1.09; Supplementary Table S2). Given the high number of missing values (77% of patients untested for *BRCA1/2*, 48% without reported ALC), we conducted a multivariable Cox regression analyses using multiple imputation of *BRCA1/2* values in the cohort that was used in the complete case analysis (n = 759). Results were unchanged with multiple imputation (Supplementary Table S3).

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Table 1. Patient demographics for all patients and for the subset with a reported ALC at any time after breast cancer diagnosis

	All patients ($n = 1,463$)	Reported ALC ($n = 759$)	No reported ALC ($n = 704$)
Age in years			
Mean (SD)	54 (±14)	53 (±14)	55 (±14)
Neighborhood socioeconomic status, statewide quintiles (1 = lowest, 5 = highest)			
Mean (SD)	4.0 (±1.2)	4.1 (±1.2)	3.8 (±1.2)
Race/ethnicity			
NH White	1,049 (72%)	543 (72%)	506 (72%)
NH Black	71 (5%)	36 (5%)	35 (5%)
NH Asian	203 (14%)	116 (15%)	87 (12%)
Hispanic	140 (9%)	64 (8%)	76 (11%)
American Joint Committee on Cancer Stage			
I	514 (35%)	255 (34%)	259 (37%)
II	714 (49%)	383 (50%)	331 (47%)
III	235 (16%)	121 (16%)	114 (16%)
Grade			
1	52 (4%)	24 (3%)	28 (4%)
2	263 (18%)	134 (18%)	129 (18%)
3	1,070 (73%)	566 (75%)	504 (72%)
Unknown	78 (5%)	35 (5%)	43 (6%)
Received neoadjuvant/adjuvant chemotherapy			
Yes	1,113 (76%)	611 (81%)	502 (71%)
No	350 (24%)	148 (19%)	202 (29%)
Received radiotherapy			
Yes	675 (46%)	338 (45%)	337 (48%)
No	788 (54%)	421 (55%)	367 (52%)
Germline *BRCA1/2* mutation status[a]			
Positive for a deleterious mutation in either gene	80 (5%)	56 (7%)	24 (3%)
Negative and/or VUS	258 (18%)	165 (22%)	93 (13%)
Untested	1,125 (77%)	538 (71%)	587 (83%)
Ever neutropenic			
Yes	142 (19%)	142 (19%)	—
No	618 (81%)	617 (81%)	—
Baseline neutropenia			
Yes	50 (10%)	50 (10%)	—
No	462 (90%)	455 (90%)	—
Minimum ALC (K/μL) postdiagnosis[b]			
Mean (SD)	0.9 (±0.6)	0.9 (±0.6)	—
Ever lymphopenic			
Yes	481 (63%)	481 (63%)	—
No	278 (37%)	278 (37%)	—
Baseline lymphopenia			
Yes	148 (29%)	147 (29%)	—
No	362 (71%)	357 (71%)	—

[a]BRCA contains an additional "untested" category.
[b]Definition of lymphopenia: <1 K/μL.

ALC, OM, and BCM over time

Higher ALC was associated with lower OM, with an ALC–time interaction of borderline statistical significance ($P = 0.06$), implying that the magnitude of ALC/OM association may increase with time. A significant association was seen in the BCM model: at diagnosis, a 1 K/μL increase in ALC had an OR of 0.16 (95% CI, 0.08–0.32). One year postdiagnosis (year 2), this OR was 0.14 (CI, 0.08–0.26). This significant inverse association of ALC and BCM continued monotonically through the sixth year postdiagnosis (OR = 0.10; CI, 0.04–0.21; Table 5). Furthermore, temporal analyses for the radiotherapy and neutropenia variables were performed and showed that having radiotherapy after having a minimum ALC measurement (vs. not having radiotherapy) was significantly associated with reduced mortality (HR = 0.12; 95% CI, 0.05–0.30), whereas radiotherapy before a minimum ALC value (vs. no radiotherapy) was not significantly associated with mortality. There was no evidence to suggest that the timing of neutropenia relative to the timing of the minimum ALC measurement had a protective effect (Supplementary Tables S4–S7).

Exploratory analysis of TILs, ALC, and other lymphocyte counts

For the 70 neoadjuvant chemotherapy trial participants with pretreatment stromal TIL information, TILs had a borderline positive association with pretreatment ALC ($P = 0.05$, $\rho = 0.25$), but not pretreatment percent peripheral lymphocytes ($P = 0.49$, $\rho = 0.08$). In contrast, there was a statistically significant positive association between TILs and the minimum percent lymphocyte value collected at any time including during neoadjuvant chemotherapy ($P = 0.02$, $\rho = 0.28$, Supplementary Fig S2; Supplementary Fig S3 shows a comparison between minimum ALC and minimum lymphocyte percent). An exploratory analysis of pathologic complete response (pCR) to neoadjuvant chemotherapy in 60 of the clinical trial patients with both TIL and response data available showed no significant association between minimum ALC value ($P = 0.62$) or ever having lymphopenia ($P = 0.53$) and achieving pCR to neoadjuvant chemotherapy (data not shown).

Published OnlineFirst March 26, 2018; DOI: 10.1158/1078-0432.CCR-17-1323

Table 2. Multivariable analysis of predictors of lymphopenia (ALC <1 K/μL; $n = 747$)

	OR (95% CI)
Neighborhood socioeconomic status (vs. 1 = lowest)	1.06 (0.88–1.28)
NH Black (vs. NH white)	0.50 (0.14–1.43)
NH Asian (vs. NH white)	1.02 (0.58–1.76)
Hispanic (vs. NH white)	1.20 (0.52–2.63)
Positive for deleterious mutation in *BRCA1/2*[a] (vs. negative/VUS)	1.11 (0.50–2.42)
Age at diagnosis (per year)	0.99 (0.98–1.01)
Stage II (vs. stage I)	0.72 (0.46–1.12)
Stage III (vs. stage I)	**1.84 (1.03–3.28)**
Grade 2 (vs. grade 1)	2.18 (0.55–14.58)
Grade 3 (vs. grade 1)	3.64 (0.99–23.52)
Unknown grade (vs. grade 1)	2.06 (0.37–16.19)

NOTE: Statistically significant variables are in bold font.
[a]*BRCA1/2* contains an additional "untested" category.

Table 3. Multivariable analysis of the association between minimum ALC and OM ($n = 747$)

	HR (95% CI)
Minimum ALC	**0.23 (0.16–0.35)**
Neoadjuvant/adjuvant chemotherapy use (vs. none)	**0.53 (0.34–0.84)**
Radiotherapy use (vs. none)	0.75 (0.54–1.03)
Neighborhood socioeconomic status (vs. 1 = lowest)	**0.85 (0.74–0.97)**
NH Black (vs. NH white)	1.72 (0.91–3.24)
NH Asian (vs. NH white)	0.98 (0.62–1.54)
Hispanic (vs. NH white)	1.29 (0.75–2.23)
Positive for deleterious mutation in *BRCA1/2* (vs. negative/VUS)	0.87 (0.40–1.91)
Untested for deleterious mutation in *BRCA1/2* (vs. negative/VUS)	1.45 (0.91–2.32)
Age at diagnosis (per year)	1.01 (1.00–1.03)
Ever neutropenic (ANC <1 K/μL) (vs. never neutropenic)	1.06 (0.72–1.55)
Stage II (vs. stage I)	1.47 (0.97–2.23)
Stage III (vs. stage I)	**4.25 (2.69–6.74)**
Grade 2 (vs. grade 1)	2.58 (0.60–11.05)
Grade 3 (vs. grade 1)	3.82 (0.93–15.72)
Unknown grade (vs. grade 1)	2.18 (0.46–10.35)

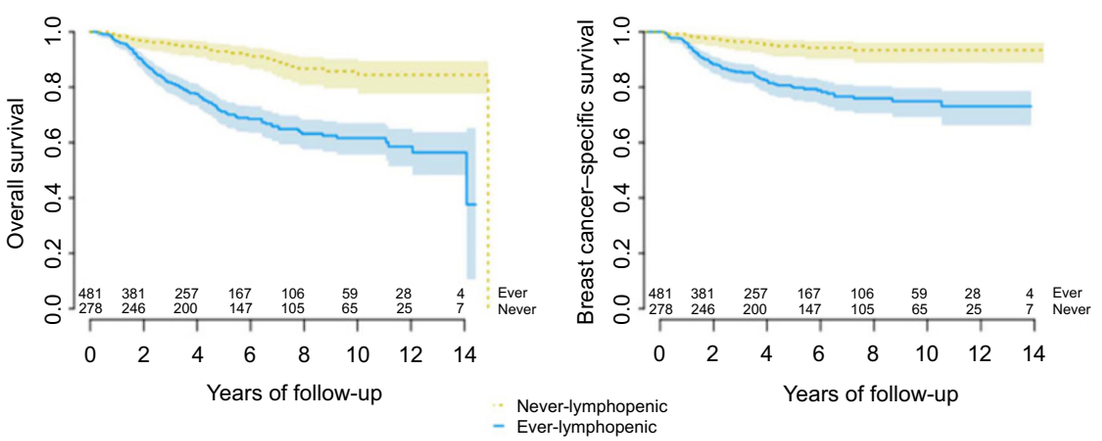
NOTE: Statistically significant variables are in bold font.

Sensitivity analysis

Of 532 patients with percent lymphocyte counts available, higher percent lymphocyte counts were associated with lower BCM and OM (HR = 0.91; 95% CI, 0.88–0.95 and HR = 0.92; 95% CI, 0.89–0.94, respectively; Supplementary Tables S8 and S9). Because more patients had ALC data available ($n = 747$, compared with 532 with percent lymphocyte count data), we present the ALC analysis primarily. To investigate the possibility that metastatic recurrence (and subsequent chemotherapy) caused the observed association between higher lymphocyte counts and lower mortality, we restricted ALC values to the first year postdiagnosis, when metastatic recurrence is rare. We observed the same inverse association of minimum ALC with BCM and OM when we restricted ALC values to the first year postdiagnosis, which offers evidence that a low ALC due to treatment of metastatic recurrence does not explain the observed finding of higher BCM and OM. Furthermore, we defined the term "prechemotherapy" ALC as an ALC value measured within 3 months of breast cancer diagnosis. We found the same inverse association of minimum ALC with OM but not with BCM with this restricted sample size [512 patients had a baseline measure of ALC within the first 3 months of diagnosis, and 29 patients (6%) met the criteria of lymphopenia (ALC <1.0 K/μL; Supplementary Figs. S4–S6]. To address the temporal relationship between laboratory measures and neoadjuvant/adjuvant chemotherapy receipt, we created indicator variables in our survival models for the time order of ALC and chemotherapy. The time sequence variable was not significantly associated with BCM or OM (data not shown).

Discussion

We found that peripheral ALC postdiagnosis was a strong predictor of BCM and OM among a large cohort of patients with stages I–III TNBC. Notably, this inverse association between ALC



Note: Drop in never-lymphopenic overall survival is due to death of the patient with the longest follow-up time. The shaded areas represent the CIs for each curve.

Figure 1.
Kaplan–Meier curves showing overall survival and breast cancer–specific survival as a function of ALC (lymphopenia defined as ALC <1 K/μL).

Published OnlineFirst March 26, 2018; DOI: 10.1158/1078-0432.CCR-17-1323

Table 4. Multivariable analysis of the association between minimum ALC and BCM ($n = 747$)

	HR (95% CI)
Minimum ALC	**0.19 (0.11–0.34)**
Neoadjuvant/adjuvant chemotherapy use (vs. none)	0.54 (0.29–1.01)
Radiotherapy use (vs. none)	0.70 (0.46–1.06)
Neighborhood socioeoconomic status (vs. 1 = lowest)	0.85 (0.71–1.00)
NH Black (vs. NH white)	1.54 (0.66–3.55)
NH Asian (vs. NH white)	0.78 (0.42–1.44)
Hispanic (vs. NH white)	1.35 (0.71–2.56)
Positive for deleterious mutation in *BRCA1/2*[a] (vs. negative/VUS)	0.98 (0.36–2.64)
Untested for deleterious mutation in *BRCA1/2*[a] (vs. negative/VUS)	1.91 (1.01–3.59)
Age at diagnosis (per year)	0.99 (0.98–1.01)
Ever neutropenic (ANC <1 K/μL; vs. never neutropenic)	1.01 (0.63–1.62)
Stage II (vs. stage I)	**2.61 (1.37–4.97)**
Stage III (vs. stage I)	**7.59 (3.83–15.04)**
Grade 2 (vs. grade 1)	2.27 (0.29–17.66)
Grade 3 (vs. grade 1)	3.06 (0.41–22.69)
Unknown grade (vs. grade 1)	1.08 (0.11–10.65)

NOTE: Statistically significant variables are in bold font.
[a]*BRCA1/2* contains an additional "untested" category.

and mortality appeared to strengthen over time: in the sixth year postdiagnosis, there was an absolute difference of >10% in BCM among everlymphopenic versus neverlymphopenic TNBC patients (15% and 4%, respectively). The increasing separation of mortality curves over time might be consistent with an immune effect that enhances response to initial therapy of TNBC. Moreover, in a cohort of TNBC patients treated on a prospective clinical trial with available TIL data, there was a direct correlation between higher TILs and higher peripheral lymphocyte counts during neoadjuvant chemotherapy.

To our knowledge, this is the first study to demonstrate a significant inverse association between peripheral lymphocyte counts and cancer-specific mortality in patients with potentially curable TNBC. Among breast cancer subtypes, TNBC has the worst prognosis, yet also the strongest evidence for an effect of TILs on survival (9, 11, 12). Two recent studies showed that with every 10% increase in stromal TILs, there was an associated reduction in the risk of TNBC relapse or death (35). Taken together with recent TIL studies, the current results strengthen the case for ongoing trials of PD-1, PD-L1, and other immunotherapies in TNBC (16, 19). Furthermore, this study raises the question as to whether strategies to protect lymphocytes from chemotherapy in the adjuvant setting might be beneficial. On multivariable analysis, the main predictor of developing lymphopenia was neoadjuvant/adjuvant chemotherapy use. Prospective studies should be conducted to characterize the development of and risk factors for lymphopenia, in addition to its association with the presence of TILs and the tumor response to therapy.

Table 5. Unadjusted association between ALC, time in years since breast cancer diagnosis, and OM and BCM

ALC over time	OM ($n = 747$) OR (95% CI)	BCM ($n = 747$) OR (95% CI)
Diagnosis to 12 months (year 1)	0.34 (0.23–0.49)	0.16 (0.08–0.32)
12–24 months (year 2)	0.30 (0.22–0.42)	0.14 (0.08–0.26)
25–36 months (year 3)	0.27 (0.20–0.37)	0.13 (0.07–0.22)
37–48 months (year 4)	0.24 (0.18–0.33)	0.12 (0.07–0.21)
49–60 months (year 5)	0.21 (0.15–0.31)	0.11 (0.05–0.20)
61–72 months (year 6)	0.19 (0.13–0.29)	0.10 (0.04–0.21)

This study adds a piece to the intriguing puzzle of how the immune system mediates treatment response and cure in TNBC. Immunoediting, the concept of tumor recognition by the host immune system, host protection against the tumor, and tumor escape (36–38), has garnered more recognition with recent advances in immunotherapy (14–17). It is increasingly evident that therapy-associated lymphocyte depletion in breast cancer patients can be associated with opportunistic infections, such as *Pneumocystis carinii* pneumonia (39–41), similar to other immunocompromised hosts, such as those on long-term corticosteroid use or with acquired immunodeficiency syndrome (42–45). One emerging theory is that tumors with greater mutational burden are more immunogenic and thus respond better to drugs that target the interplay between host and tumor immune responses (15, 46). Most TNBCs have basal-like subtype on gene expression profiling and high histologic grade, possibly denoting greater genomic instability in comparison with hormone receptor–positive breast cancers (13, 47). Furthermore, approximately 20% of TNBC patients have a deleterious germline *BRCA1/2* mutation, which is an inherited defect in DNA double-strand repair that causes tumor genomic instability (48, 49). One quarter of tested patients in the current study carried a *BRCA1/2* mutation, but we did not observe a significant interaction between *BRCA1/2* mutations and ALC on mortality. However, this null result might relate to low genetic testing rates in this 2000 to 2014 diagnosis cohort: More than 70% were untested according to Myriad Genetics, the only laboratory that provided clinical *BRCA1/2* testing during most of the study period. The low testing rate reflects changes in understanding of the genetic causes of TNBC over time, as *BRCA1/2* testing of all TNBC patients diagnosed at age <60 was not widely recommended until the later years of the study period. Moreover, recent work has shown that several other DNA repair gene mutations are associated with TNBC and might cause a similar genomic instability phenotype as with *BRCA1/2* (48). Future studies of TNBC patients tested for germline *BRCA1/2* and other DNA repair gene mutations, in addition to genomic instability assays such as the homologous recombination deficiency assay (29, 50), will be essential to answer questions about immune function and mortality in TNBC.

Aspects of this study warrant consideration. Limitations include its retrospective design and restrictions inherent to using clinical information that was not originally collected for research purposes, notably missing data. In particular, we cannot rule out the possibility that patients with poorer prognosis more often had blood counts measured. However, it is reassuring that low ANC was not associated with higher mortality, suggesting that the observed results are lymphocyte specific. Furthermore, a sensitivity analysis restricted to ALC values measured within the first year postdiagnosis (a time period before most metastatic recurrences) showed consistent associations with OM and BCM. We addressed missing data using multiple imputation methods, which yielded similar results as complete case analysis. The Oncoshare database focuses on a single geographic area with disproportionately high numbers of NH Whites and Asians and thus may not fully represent all TNBC patients. Fortunately, providers across different health care settings used the same EMR: Thus, laboratory values and reference ranges were consistent across sites and did not constitute a source of bias. Random error may have

Published OnlineFirst March 26, 2018; DOI: 10.1158/1078-0432.CCR-17-1323

contributed to the reported associations being underestimates of the true values. The intriguing temporal relationship between radiotherapy and minimum ALC measurements warrants further study, specifically evaluating the duration of lymphopenia, which we were unable to do using our current data source. Furthermore, additional research elucidating the temporal relationship between chemotherapy, radiotherapy, and lymphopenia is warranted to understand the range of determinants associated with these findings. As with any observational study, causality inferred from associations can be misleading due to uncontrolled selection bias or confounding. Although provocative, the correlation we observed between TILs and lymphocyte counts during neoadjuvant therapy is derived from an exploratory analysis and thus must be considered hypothesis-generating only.

This study's limitations are offset by its considerable strengths: the large number of TNBC patients (>1,400); long-term, highly complete mortality data available through SEER database linkage; detailed laboratory results available from EMRs and a genetic testing laboratory; and the real-world scope of more than one health care system, including an academic referral center and a community-based, multisite practice.

In summary, we report a substantial, specific, and lasting inverse association between peripheral lymphocyte counts and cancer-specific and overall mortality among women with potentially curable TNBC. These results add to an emerging understanding of immune function in TNBC. In future clinical trials for TNBC, peripheral lymphocyte counts should be explored in connection with TILs as a potential biomarker for treatment efficacy and survival.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

The ideas and opinions expressed herein are those of the authors, and endorsement by the University or State of California, the California Department of Health Services, the NCI, or the Centers for Disease Control and Prevention, or their contractors and subcontractors is not intended nor should be inferred.

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Acknowledgments

The authors gratefully acknowledge research support from the Susan and Richard Levy Gift Fund, the Suzanne Pride Bryan Fund for Breast Cancer Research, the Breast Cancer Research Foundation, the Susan G. Komen for the Cure Foundation, the Jan Weimer Junior Faculty Chair in Breast Oncology, the Regents of the University of California's California Breast Cancer Research Program (16OB-0149 and 19IB-0124), the Stanford University Developmental Research Fund, the BRCA Foundation, and the NCI's Surveillance, Epidemiology, and End Results Program under contract HHSN261201000140C awarded to the Cancer Prevention Institute of California. The project was supported by NIH CTSA award UL1 RR025744. The collection of cancer incidence data used in this study was supported by the California Department of Health Services as part of the statewide cancer reporting program mandated by California Health and Safety Code Section 103885; the NCI's Surveillance, Epidemiology, and End Results Program under contract HHSN261201000140C awarded to the Cancer Prevention Institute of California, contract HHSN261201000035C awarded to the University of Southern California, and contract HHSN261201000034C awarded to the Public Health Institute; and the Centers for Disease Control and Prevention's National Program of Cancer Registries, under agreement #1U58 DP000807-01 awarded to the Public Health Institute. The authors thank Anne-Renee Hartman, MD, and Brian Allen, MS, C.G.C., of Myriad Genetics for provision of genetic testing data and Harold S. Luft, PhD, coprincipal investigator of the Oncoshare Project and Director of the Palo Alto Medical Foundation Research Institute.

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Received May 9, 2017; revised October 23, 2017; accepted March 20, 2018; published first March 26, 2018.

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Published OnlineFirst March 26, 2018; DOI: 10.1158/1078-0432.CCR-17-1323

Clinical Cancer Research

Higher Absolute Lymphocyte Counts Predict Lower Mortality from Early-Stage Triple-Negative Breast Cancer

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Clin Cancer Res 2018;24:2851-2858. Published OnlineFirst March 26, 2018.