

Total White Blood Cell Count, Absolute Neutrophil Count, Absolute Lymphocyte Count, Neutrophil-to-Lymphocyte Ratio and the Risk of Breast Cancer: The NHANES I Epidemiologic Follow-up Study

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Abstract: The purpose of this study was to examine total white blood cell count (TWBCC), absolute neutrophil count (ANC), absolute lymphocyte count (ALC), and neutrophil-to-lymphocyte ratio's (NLR) association with the risk of incident breast cancer (BCa). A prospective analysis was conducted among a cohort of 2,873 women from the First National Health and Nutrition Examination Survey (NHANES I) Epidemiologic Follow-up Study. In the present analysis, the association found between lower ALC and increased BCa risk was no longer statistically significant after adjusting for age. Additionally, no statically significant associations were found between any level of ANC, NLR, and TWBCC with incident BCa. Therefore, additional studies, which address the limitations encountered in the present analysis, are needed to validate, or rule out, their use as biomarkers of BCa risk in women.

Keywords: Breast Cancer, Lymphocyte Count, Neutrophil Count, Neutrophil-to-Lymphocyte Ratio Risk, White Blood Cell

1. Introduction

Breast cancer (BCa) is the most common, and burdensome, malignancy among women in the United States (U.S.). It is estimated that 3 million women in the U.S. are living with BCa [1]. Over the past 30 years, female BCa incidence rates in the U.S. have steadily increased from an estimated 105 new BCa cases per 100,000 women in 1975 to approximately 130 new BCa cases per 100,000 women in 2012 [1]. Although BCa mortality rates have decreased in this same period (30 deaths per 100,000 women in 1975 to 21 deaths per 100,000 women in 2012), rates of this disease continue to rank second out of the top ten cancer death rates in the U.S. [1, 2].

The observed decreases in BCa mortality among women in the U.S. have been attributed mainly to a combination of early detection screening and improved BCa treatment [3, 4, 5]. However, BCa yet remains one of the most significant illnesses among U.S. women [1, 2], indicating a need for

further improvements in both screening and treatment strategies. The identification of new biomarkers for BCa might provide a new path for both improved early diagnosis, as well as improved treatment strategies for this deadly disease [6].

Inflammation has been identified as a characteristic of several cancer types and is often characterized by the presences of various white blood cell (WBC) subtypes in the tumor microenvironment [7, 8, 9, 10, 11]. As a result, peripheral WBC's might serve as an indicator of tumorigenesis. A significant body of evidence suggests that absolute counts of peripheral WBC's [12], to include lymphocyte count [13, 14], neutrophil count [15], and neutrophil-to-lymphocyte ratio [16, 17, 18] serve as significant predictors BCa prognosis. However, their use as predictors of BCa onset has not yet been established. To date, few studies have examined peripheral WBC's relationship with the risk of BCa [12, 19, 20]. Of these few, results remain mixed [12, 19, 20]. Therefore, this study was

conducted to examine TWBCC, absolute neutrophil count (ANC), absolute lymphocyte count (ALC), and neutrophil-to-lymphocyte ratio's (NLR) association with the risk of incident BCa in a cohort of adult females from the First National Health and Nutrition Examination Survey (NHANES I) Epidemiologic Follow-up Study.

2. Methods

2.1. The NHANES I Epidemiologic Follow-up Study

The NHANES I Epidemiologic Follow-up Study (NHEFS) was a longitudinal analysis conducted as a collaborative project between the National Institute on Aging and the National Center for Health Statistics [21]. The primary purpose of the NHEFS was to examine the relationships between clinical, nutritional, and behavioral factors obtained during the NHANES I with resultant morbidity, mortality, hospital utilization, functional limitation, institutionalization, and changes in risk factors. The NHEFS cohort included 14,407 adults, aged 25-74 years, who completed a medical examination in the NHANES I, during 1971-1975. To date, a total of four follow-up studies have been conducted for the NHEFS cohort. The first wave of follow-up was conducted 1982-1984, the second wave in 1986, the third wave in 1987, and the fourth wave in 1992 [21]. A total of 96% of NHEFS study participants have been successfully traced at some point through the fourth follow-up wave in 1992 [21]. Additional information regarding the NHEFS study design is described in detail elsewhere [21]. Data files for the NHEFS are de-identified and are available for public use [22, 23].

2.2. Study Participants

A total of 8,596 women participated in the NHEFS. Initially, 3,065 women, which reported not to have any known tumor or malignancy at baseline, were identified for the present analysis. This sample of women was then evaluated to determine if they provided information regarding TWBCC, differential WBC count (DWBCC), as well as their responses for the potential confounding variables of age, physical activity status, smoking, and menopause status at baseline. Women providing this information at baseline were then followed-up through the fourth wave of the NHEFS to determine their BCa status. Women, who did not provide, or were missing, data pertaining the variables of interest, were excluded from this study. After applying inclusion-exclusion criteria, a total of 2,873 women were available for the analytic cohort.

2.3. White Blood Cell Counts

Laboratory methods that were used to obtain and analyze blood samples from participants of the NHANES I are described in detail elsewhere [24]. In the NHANES I, TWBCC was initially provided in cells per cubic millimeter (cells/ mm^3). Differential WBC counts obtained from the same study participants were provided in the percentage of 100 cells. For the present analysis, TWBCC was converted to

cells per microliter (cells/ μL). Absolute neutrophil count and ALC were also converted to cells/ μL by taking the product of the provided WBC subtype percentage and the TWBCC for each respondent. Neutrophil-to-lymphocyte ratio was estimated by taking the quotient of ANC and ALC. Finally, quintiles of TWBCC, ANC, ALC, and NLR were then created, based on their quantile distribution in the analytic cohort.

2.4. Breast Cancer Incidence

Breast cancer incidence was the outcome of interest assessed in the present analysis. In the NHEFS, BCa cases were identified using a combination of follow-up interviews and death certificate information. For interviews, BCa status was determined using in-person interviews conducted during the NHEFS 1982-84 follow-up period, and by Computer Assisted Telephone Interview (CATI) for 1986, 1987, and 1992 follow-up periods [25]. A total of 107 BCa cases were identified during follow-up interviews. Two additional BCa cases were identified by review of death certificates, for a total of 109 BCa cases.

2.5. Covariates

Baseline age, race, physical activity, smoking, and menopause status were assessed as potential confounders of the WBC-BCa relationship in this analysis. Concerning study participant race, women were classified, at baseline, as either white, black, or other. Physical activity status was ascertained by asking women at baseline, "In your usual day, aside from recreation, how active are you?" [22]. Resultant physical activity status was categorized as "very active", "moderately active" or "quite inactive" [22]. Smoking status was ascertained by asking women at baseline, "Have you smoked at least 100 cigarettes during your entire life?" [22]. Finally, menopause status was obtained by asking women at baseline if their menstrual cycle had completely stopped [22].

2.6. Statistical Analysis

Cox proportional hazards regression analyses were conducted to examine the relationship between baseline categories of TWBCC, ANC, ALC, and NLR with incident BCa. Both crude and adjusted models were constructed to estimate the relative risk (RR), and 95% confidence intervals (CI) for each quintile of baseline TWBCC, ANC, ALC, and NLR. Multivariate models were adjusted for baseline age, race, physical activity, smoking, and menopause status. A test for trend across quintiles was conducted by assigning the median values for each quintile of TWBCC, ANC, ALC, and NLR, followed by an evaluation of these values as a continuous variable in separate Cox regression models. Resulting *P*-values were reported.

Follow-up time for BCa cases was estimated as the number of years, from the year of the initial NHANES I exam, to the date of diagnosis, interview date, or date of death. For study participants without BCa, follow-up time was defined as the number of years, from the initial

NHANES I exam to the subject's date of the last interview, date of death, or date last known to be alive. The mean follow-up time for the analytic cohort was 15.9 years. Finally, visual inspection of the log (-log) survival curves was used to test the assumption of proportionality. Statistical Package for Social Sciences (SPSS) Version 21.0® was used for all analyses.

3. Results

Table 1 presents a summary of population characteristics by BCa status. The mean age among women with incident BCa was 50.6 years (standard deviation [SD] = 12.9 years), compared to 47.4 years (SD= 14.1 years) among women without BCa. By race, 91.7% of incident BCa occurred among white women, compared to 8.3% of black women. Concerning physical activity, at least 90% of women reported to be moderate to very active. Approximately 47% of women with incident BCa reported smoking at least 100 cigarettes in their lifetime while almost 59% having reached menopause. Finally, a lower mean TWBCC and ALC was observed among women with incident BCa, compared to women without BCa. To the contrary, a higher mean ANC and NLR was found in women with BCa, compared to those not reporting to have BCa (Table 1).

Table 1. Population Baseline Characteristics by Breast Cancer Status (N=2,783).

	Yes BCa (N=109)	No BCa (N=2,764)
Characteristic		
Age (years), mean (SD)	50.6 (12.9)	47.4 (14.1)
Race, %		
White	91.7	85.7
Black	8.3	13.3
Other	0.0	1.0
Physical Activity, %		
Very Active	39.4	40.3
Moderately Active	51.4	49.2
Quite Inactive	9.2	10.5
Ever Smoke, %		
Yes	46.8	45.8
No	53.2	54.2
Menopause, %		
Yes	58.7	51.6
No	41.3	48.4
TWBCC (cells/ μ L), mean (SD)	7,334.9 (1,792.3)	7,389.2 (1,975.8)
ANC (cells/ μ L), mean (SD)	4,455.5 (1,512.7)	4,361.5 (1,556.0)
ALC (cells/ μ L), mean (SD)	2,551.7 (786.3)	2,664.3 (909.7)
NLR (cells/ μ L), mean (SD)	1.9210 (0.9772)	1.8164 (1.0555)

Abbreviations: ALC, absolute lymphocyte count; ANC, absolute neutrophil count; BCa, breast cancer; NLR, neutrophil-to-lymphocyte ratio; SD, standard deviation; TWBCC, total white blood cell count; μ L, microliter

Cox regression analyses results are presented in Table 2. In an unadjusted model, women with an ALC of 2,747-3,354 cells/ μ L had a 1.9-fold increased risk of BCa (RR 1.92, 95% CI 1.03-3.58), compared to women in the highest ALC quintile. However, this association was no longer statically significant after adjusting for age (RR 1.87, 95% CI 1.00-3.48). Further multivariate adjustment slightly attenuated this

association (RR 1.85, 95% CI 0.99-3.45) but, this association remained statistically insignificant. In a similar multivariate adjusted model, a 1.7-fold increased risk of BCa was observed in women with an ALC \leq 1,926 cells/ μ L, compared to those of the highest ALC quintile. However, this association was also not statistically significant (RR 1.71, 95% CI 0.91-3.23).

In both age and multivariate adjusted models, inverse associations with incident BCa were observed among women in lower ANC quintiles, compared to those in the highest quintile of ANC. However, none of these associations were statistically significant (Table 2). Inverse associations with BCa risk were also observed in all models among women in the NLR range of 1.8001-2.2900 when compared to those of the highest NLR quintile. However, these associations were also not statistically significant (Table 2). No statistically significant associations were found between any quintile of TWBCC and risk of incident BCa (Table 2).

Table 2. Relative Risk of Breast Cancer Incidence by TWBCC, ANC, ALC, and NLR Quintile.

	RR	95% CI	RR*	95% CI	RR**	95% CI
TWBCC (cells/ μ L)						
\geq 8,902	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
7,602-8,901	1.38	0.75-2.55	1.34	0.73-2.48	1.37	0.74-2.53
6,702-7,601	1.33	0.71-2.48	1.24	0.67-2.32	1.27	0.68-2.38
5,702-6,701	1.08	0.56-2.06	0.99	0.52-1.89	1.03	0.53-1.99
\leq 5,701	1.16	0.62-2.19	1.06	0.56-1.99	1.15	0.60-2.19
P for trend	.924		.780		.995	
ANC (cells/ μ L)						
\geq 5,453	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
4,526-5,452	0.82	0.44-1.50	0.78	0.42-1.43	0.79	0.43-1.45
3,830-4,525	1.07	0.61-1.89	0.98	0.56-1.72	1.00	0.57-1.78
3,102-3,829	0.86	0.47-1.56	0.77	0.42-1.40	0.80	0.44-1.47
\leq 3,101	0.93	0.52-1.67	0.84	0.47-1.52	0.93	0.51-1.69
P for trend	.847		.561		.784	
ALC (cells/ μ L)						
\geq 3,355	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
2,747-3,354	1.92	1.03-3.58	1.87	1.00-3.48	1.85	0.99-3.45
2,342-2,746	1.24	0.63-2.45	1.23	0.62-2.41	1.23	0.63-2.43
1,927-2,341	1.17	0.59-2.33	1.15	0.58-2.28	1.16	0.58-2.30
\leq 1,926	1.76	0.94-3.30	1.70	0.91-3.19	1.71	0.91-3.23
P for trend	.324		.377		.365	
NLR						
\geq 2.2901	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
1.8001-2.2900	1.00	0.57-1.76	1.02	0.58-1.78	1.03	0.59-1.82
1.4738-1.8000	0.63	0.34-1.17	0.63	0.34-1.17	0.63	0.34-1.18
1.1365-1.4737	1.00	0.58-1.73	0.96	0.55-1.67	1.00	0.57-1.73
\leq 1.1364	0.76	0.42-1.37	0.73	0.40-1.32	0.78	0.43-1.42
P for trend	.397		.307		.424	

Abbreviations: ALC, absolute lymphocyte count; ANC, absolute neutrophil count; CI, confidence interval; NLR, neutrophil-to-lymphocyte ratio; RR, relative risk; TWBCC, total white blood cell count; μ L, microliter

*Adjusted for age

**Adjusted for age, race, physical activity, smoking, and menopause status

4. Discussion

The tumor microenvironment is often comprised of various types of leukocytes [7, 8, 9, 10, 11]. Because of this diverse leukocyte population in cancer-related inflammation, it has been posited that the difference between tumor

progression and elimination depend on the balance of immune modulators and mediators, produced by these WBC's [7, 8, 9, 10, 11]. As such, peripheral WBC's might serve as key biomarkers of tumorigenesis and thus, a tool for early cancer diagnosis and treatment. Despite the established role of peripheral WBC's in BCa prognosis [13, 14, 15, 16, 17, 18], their role in predicting incident BCa remains to be determined. Therefore, this study sought to determine if TWBCC, ANC, ALC, and NLR were associated with the risk of incident BCa among a cohort of women from the NHEFS.

Results from the few studies that have examined the TWBCC-BCa relationship remain inconsistent [12, 19, 20]. In one prospective analysis, involving a sample of 143,748 women, aged 50-79 years from the Women's Health Initiative, participants in the highest TWBCC quartile ($6.80-15.00 \times 10^9$ cells/L) maintained a 15% increased risk of invasive BCa (HR 1.15, 95% CI 1.04-1.26), compared to women in the lowest TWBCC quartile of $2.50-4.79 \times 10^9$ cells/L, after adjusting for age, race, physical activity, smoking, breastfeeding, family history of breast cancer, body mass index, age at menopause, history of benign breast disease, hormone use, height, parity, and bilateral oophorectomy [12]. Contrary to these findings, another prospective analysis found no significant association between any level of TWBCC and BCa risk [19]. Additionally, it is unclear if a significant association between leukocyte count and the odds of BCa was present in a recent cross-sectional study examining their association, as the results a multivariate logistic regression model including these variables of interest were not reported by the authors [20].

In the present study, no association was found between any level of TWBCC and BCa risk, when compared to women with a TWBCC of $\geq 8,902$ cells/ μ L. One possible explanation is that TWBCC serves as a significant predictor of certain types of BCa. An estimated 80-90% of incident BCa cases consist of the invasive type [26]. Margolis et al. found a significant association between increased levels of TWBCC and risk of invasive BCa [12]. In contrast, Van Hemelrijck et al. did not note the specific types of BCa present in their study population, and found no significant association with TWBCC [19]. Likewise, it was not possible to evaluate TWBCC with any specific type of BCa in the present analysis, as this information was not ascertained from women participating during the NHEFS follow-up periods.

Another possible explanation is that differences in BCa risk observed in the present analysis, versus previous studies are the result of the different TWBCC categories used. Finally, it might be possible that TWBCC serves a better predictor for other types of cancer. For example, previous studies suggest that increased levels of TWBCC are associated with the increased risk of the lung [12, 19, 27], gastric [28], and colorectal [12] cancers. Nevertheless, the current body of literature, in conjunction with the findings of this analysis, suggest that TWBCC does not serve as a significant predictor of BCa risk. Additional prospective

studies, examining TWBCC against the different types of BCa are warranted.

To date, the majority of literature, evaluating the WBC-BCa relationship, has focused primarily on BCa prognosis [13, 14, 15, 16, 17, 18, 29]. In these analyses, increased levels of peripheral neutrophils and lower levels of peripheral lymphocytes were found to be associated with increased risk of poor BCa prognosis. Specific measures used in these studies included ALC [13, 14], ANC [15], NLR [16, 17, 18], and platelet-to-lymphocyte ratio [29]. Similar to previous inquiries, this analysis used different levels of ALC, ANC, and NLR as primary measures in an attempt to understand better, their association with BCa incidence.

In the present analysis, 1.8-fold and 1.7-fold increased BCa risks were indicated in women with ALC's of 2,747-3,354 cells/ μ L and $\leq 1,926$ cells/ μ L respectively, compared to women with an ALC count of $\geq 3,355$ cells/ μ L. The T-lymphocyte response is thought to be the primary mechanism of a host's immune response to tumor onset and progression [9]. Specifically, CD8 cytotoxic T-lymphocytes (CTL), and Natural Killer T-lymphocytes (NKTL) have been identified as playing key roles in the direct killing of cancerous cells [11]. In the case of tumor escape and progression, however, an inefficient immune response is characterized in part, by the down-regulation of CD8 CTL's and NKTL's [11]. Despite this existing knowledge, it remains to be determined which specific levels of CTL's and NKTL's correspond with increased BCa risk. In the present analysis, it was not possible to evaluate these associations, as absolute counts of CTL's and NKTL's were not ascertained, during the NHANES I.

Although increased BCa risk was indicated among those with an ALC of 2,747-3,354 cells/ μ L, these associations were no longer statistically significant after adjusting for age (Table 2). Age is a well-established risk factor for BCa [30, 31]. However, increased age has also been implicated as a predictor of lower lymphocyte count and reduced lymphocyte function [32]. As such, reduced ALC levels might provide some explanation for the currently established age-BCa relationship. Nevertheless, these findings suggest that lower ALC might serve an indicator of increased BCa risk.

A significant body of literature suggests that increased ANC, as well as increased NLR, are associated with poor BCa prognosis [15, 16, 17, 18]. One cross-sectional study also reported a statistically significant association between ANC and the odds of BCa (OR 0.627, 95%CI 0.508-0.774, $P=0.001$), after multivariate adjustment [20]. Likewise, the decreased risk of incident BCa was indicated among women lower quintiles of ANC and NLR in the present analysis. However, these results were also statistically insignificant (Table 2). Although not fully understood, neutrophils have been identified as having both anti-tumor and tumor-promoting functions [33, 34]. With respect to anti-tumor functions, neutrophils are thought to secrete various chemokines and cytokines that increase immune response, allowing for the killing of tumor cells [33]. To the contrary, neutrophils contribute to the growth of tumors by cytokine and chemokine expression that suppresses an anti-tumor

immune response [34]. Although much remains to be understood about the neutrophil differentiation process in tumorigenesis, the findings of the present analysis suggest that increased ANC, as well as NLR, might serve as early indicators of BCa onset.

To the author's knowledge, the findings of this study demonstrate for the first time, an association between lower levels of ALC and incident BCa in women. This study is also among the first to examine the role of ANC, ALC, and NLR as potential predictors of BCa incidence. Additionally, the use of a prospective study design, which includes a relatively large sample size with a long follow-up period are significant strengths of the present analysis.

The results this analysis however, should be interpreted with caution, as several limitations were encountered. First, it is possible that the findings observed in this study were confounded by one or more covariates, which were not assessed. Important BCa risk factors such as family history of BCa [35], as well as a history of hormonal replacement therapy, breastfeeding history, and past oral contraceptive use [36] were not ascertained from women participants. Another significant limitation was that the majority of BCa cases were obtained via self-report. Although self-reported BCa is considered to be of high reliability [37], self-reporting bias may have resulted in misclassification of BCa status and thus, a possible over-estimation, or under-estimation of the observed results. Concerning TWBCC and DWBCC's, only one measure was ascertained (at baseline) from each NHEFS study participant. It is possible that both TWBCC and DWBCC values varied during follow-up. Finally, the results of this analysis might not be generalizable to U.S. women as a whole, as an estimated 92% of study participants were classified as white. Although incident BCa has been historically highest among non-Hispanic white women, evidence suggests that their rates are converging with BCa incidence among black women [38].

5. Conclusion

The current body of literature suggests that ANC, ALC, and NLR serve as significant predictors of BCa prognosis. However, their use as biomarkers of BCa onset and progression remain to be determined. In the present analysis, the association found between lower ALC and increased BCa risk was no longer statistically significant after adjusting for age. Additionally, no statically significant associations were found between any level of ANC, NLR, and TWBCC with incident BCa. Nevertheless, ALC, ANC, and NLR might serve as useful indicators of BCa risk in women. A better understanding of these measures in BCa risk has the potential to inform better both BCa detection and treatment strategies. Therefore, additional studies, which address the limitations encountered in the present analysis, are needed to validate, or rule out, their use as biomarkers of BCa risk in women.

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