A peripheral blood biomarker estimates probability of survival: the neutrophil–lymphocyte ratio in noncancer patients

Aim: Inflammatory biomarkers are associated with aging and disease-specific outcomes. We propose neutrophil–lymphocyte ratio (NLR) informs survival in a noncancer-bearing population. **Patients & methods:** Retrospective cohort study of patients with a noncancer diagnosis. Calculation of NLR, ascertainment of age, gender, race, cardiovascular disease and diabetes status, and association with survival was determined. **Results:** Elevated NLR was associated with worse overall survival, independent of age, gender and comorbid status. Overall survival was significantly worse for patients with high versus low NLR. **Conclusion:** Elevated NLR is associated with worse overall survival in noncancer patients. It remains unclear whether NLR reflects an acute inflammatory state, depressed host immune competence or both. NLR may simply be another predictor of survival, or potentially a modifiable risk factor.

First draft submitted: 11 April 2016; Accepted for publication: 27 June 2016; Published online: 18 August 2016

Keywords: biomarker • inflammation • neutrophil–lymphocyte ratio • noncancer • outcomes

Inflammatory response to injury and infection represents an evolved, intricate and self-regulated human defense mechanism. Lack of immune system homeostasis results in a spectrum of dysfunction ranging from immune incompetence to unimpeded inflammation. While the link between chronic inflammation and neoplasia is well recognized, immune incompetence and immunosuppression likewise engender greater susceptibility to cancer development and progression [1–3]. Systemic inflammatory biomarkers have been associated with risk of cardiovascular disease and death due to cancer. For example, peripheral white blood cell (WBC) and neutrophil counts have demonstrated ability to predict cardiovascular disease-specific mortality [4,5]. Numerous clinical investigations of neutrophil count, lymphocyte count or their ratio in cancer patients have found these biomarkers to be independent predictors of worse

overall and cancer-specific outcome [6–8]. Investigators have questioned, but not clearly answered, whether these markers of inflammation are a cause or result of the disease state.

Although the biological relevance of some biomarkers is poorly understood, the causative effects of inflammation on an otherwise normal host offer the possibility for intervention or modification. The inexpensive and accessible ratio of neutrophils-to-lymphocytes (NLR) in peripheral blood is a biomarker associated with cancer recurrence and survival in many different malignancies. We hypothesized NLR is a biomarker associated with overall survival in noncancer-bearing patients. The aims of this study were to describe the differences in NLR associated with age in patients without cancer, and to determine the association of NLR with patient factors and overall survival.

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Patients & methods

This is a retrospective cohort study approved by the institutional review board. A query of the electronic health record was conducted for all patients evaluated at a single institution beginning in January 1998. Study entry required at least one complete blood count (CBC) with differential and a noncancer diagnosis. If more than one complete blood count was available, results from the earliest laboratory date were used. International Classification of Diseases, ninth revision, (ICD-9) codes were used to exclude patients with cancer diagnoses (ICD: 140–209 and 230–239). Date of birth, gender and race were compiled along with concomitant diagnoses of cardiovascular disease (ICD: 410–414.9, 429.2) and diabetes mellitus (ICD: 249–250.9).

Ascertainment of vital status was conducted by weekly electronic transmission from the Social Security Death Master File. Because of biases in death reporting by individual states in recent years, the study period included patients evaluated through October 2011. Additional death information recorded by medical staff with direct information on patient status was submitted to the patient registration system.

CBCs were made using automated cell counters in a Clinical Laboratory Improvement Amendments certified institutional laboratory. Patients with WBC values outside the adult reference range (4.0–11.0 k/μl) were excluded so as not to include patients with dis-

orders of immune suppression or infectious etiology (Supplementary Figure 1). The ratio of absolute neutrophil count (ANC) to absolute lymphocyte count (ALC) was used to generate the NLR. Values of zero for ANC or ALC were excluded. The median value of NLR for the cohort was used to define high and low NLR groups. Comparisons were made using X² test and *t*-test depending on categorical or continuous variables. Survival data were left truncated and right censored, with left-truncation age as date of blood count and right censoring as either age at death or at last follow-up. Since age was the left-truncation point it was not included in the multivariable model.

Patients were not engaged in setting the research agenda for this study.

Results

During the study period, 28,968 patients who met inclusion criteria of noncancer diagnosis at time of presentation and normal WBC were evaluated at our institution. The median age at determination of NLR was 52 years (range <1–103) with 67% female patients and majority (83%) white race. The prevalence of cardiovascular disease and diabetes mellitus was 6 and 7%, respectively. The median NLR of the entire cohort was 2.27. At last follow-up, 9% patients (n = 2625) had died of any cause. To determine differences in patient factors based on NLR, the median NLR was used to define high (>2.27) and low (<2.27) groups (Table 1). Patients

Table 1. Factors associated with high and low neutrophil–lymphocyte ratio.			
Factors	Low NLR [†] (n = 14,446)	High NLR [†] (n = 14,522)	p-value
Age at NLR, mean (years)	48.3	54.5	<0.0001 [‡]
Gender, number (%):			0.3669 [§]
– Female	10,145 (70)	9365 (64)	
– Male	4301 (30)	5157 (36)	
Ethnicity, number (%):			0.2568 [§]
– White	11,522 (80)	12,756 (88)	
– Black	1523 (11)	644 (4)	
– Asian	863 (6)	596 (4)	
– Other	538 (4)	526 (4)	
Cardiovascular disease, number (%)			0.3521 [§]
– No	13,875 (96)	13,472 (93)	
– Yes	571 (4)	1050 (7)	
Diabetes mellitus, number (%)			0.7883 [§]
– No	13,487 (93)	13,336 (92)	
– Yes	959 (7)	1186 (8)	
[†] Low NLR: <2.27; high NLR: >2.27. [‡] X ² test. [§] X ² test for proportions. NLR: Neutrophil–lymphocyte ratio.			

Table 2. Factors associated with overall survival by multivariate analysis.

	Hazard ratio	95% CI	p-value
NLR (high vs low)	1.075	1.067–1.082	<0.0001
Gender (male vs female)	1.745	1.614–1.887	<0.0001
Diabetes mellitus (yes vs no)	1.622	1.461–1.801	<0.0001
Cardiovascular disease (yes vs no)	1.371	1.234–1.523	<0.0001

NLR: Neutrophil–lymphocyte ratio.

with high NLR had a higher median age than those with a low NLR. The proportion of male patients was not significantly greater in the high NLR group; however, male patients had higher median NLR compared with female patients (2.40 vs 2.23). There were no differences in NLR according to race, cardiovascular disease and diabetes mellitus status. The altered ratio of average ANC and ALC resulted in a composite NLR that appeared to differ when patient age was categorized according to decade (Figure 1). Median overall survival was significantly worse for patients with high versus low NLR (log rank, $p < 0.0001$) (Figure 2). The estimated 10-year survival for patients with low and high NLR was 94.7 and 87.6%, respectively. Factors associated with worse overall survival on univariate analysis included increasing age, male gender, elevated NLR, cardiovascular disease and diabetes (data not shown). Multivariate analysis confirmed association of elevated NLR with worse overall survival independent of male gender, cardiovascular disease and diabetes mellitus status (Table 2).

Discussion

The current study demonstrated an elevated NLR in patients without cancer was associated with overall survival independent of age, gender, cardiovascular disease and diabetes mellitus. While WBC remained static over the entire age range, the changing ratio of neutrophils to lymphocytes manifested as an inexorable rise in NLR across patient ages spanning ten decades. While the data in noncancer patients presented here echo previous findings in patients with cancer, our novel observations suggest NLR may provide additional insight into the dynamic process of immune cell variation associated with aging [9].

The association of elevated WBC and neutrophils with cardiovascular disease and all-cause mortality has been demonstrated in other populations. Proponents of the Glasgow Prognostic Score in cancer patients, Proctor and colleagues investigated the relationship between inflammatory markers and all-cause mortality [10]. Levels of C-reactive protein, albumin, neutrophil count, lymphocyte count and platelet count were each associated with all-cause and cancer-related mortality. Although their report utilized a previously established scoring tool, the relationship of neutrophil and

lymphocyte counts with age was not addressed. Even so, our findings are consistent with those from Glasgow and an earlier study from the Multiple Risk Factor Intervention Trial in which systemic inflammation was linked with decreased survival [11].

Models of the permissive and suppressive nature of specific myeloid cell subsets demonstrate the necessary balance between immune cell compartments [12]. Subtle changes in immune system homeostasis characterized by a changing NLR may govern susceptibility or resiliency to infection and invasion (i.e., mutated self and cancer). Although changing leukocyte quantities can result from environmental pressures, they are known also to reflect functional alterations attributable to genetic variation [13]. Heterogeneity of immune cell subsets within a population will only be defined with longitudinal studies of both healthy and sick patients [14]. Concurrent and comprehensive study of phenotype and function of immune cell subsets may also help characterize disease susceptibility via permissive, age-related immune dysfunction.

Limitations

Retrospective analysis and lack of inclusion of cause of death are noteworthy limitations of this study. Also lacking is information regarding subsequent develop-

Figure 1. Mean absolute neutrophil (blue) and absolute lymphocyte (green) counts according to patient age by decade are shown with overlying mean neutrophil–lymphocyte ratio (red line, with standard error of the mean).

Figure 2. Estimated overall survival according to high (orange) and low (blue) neutrophil–lymphocyte ratios.

ment of cancer, the use of immunosuppressive medications and coincident autoimmune disorders. This study is also deficient in population heterogeneity representative of larger cross-sectional studies. Despite no known cancer diagnoses present at the time of CBC in this cohort, we have no information why blood tests were performed. Incidental measurement of NLR was used in this study, whereas the change in NLR over time could prove more valuable. Evidence for age-associated changes in innate immunity supports the utility of functional analyses; therefore, prospective evaluation of cell genotype and function is warranted [9,15]. The challenge of our findings, and others, is the capacity to causally link any biomarker to death or disease, and thereby distinguish it as a therapeutic target [16]. Indeed, NLR is not a disease-specific biomarker; hence, its prognostic value in diverse populations underscores the intense pursuit of the mechanistic underpinnings of age- and disease-associated inflammation. An integrated systems approach is required to appreciate the interplay of aging, dysregulated immune function and disease development [17,18].

Conclusion

The ratio of neutrophils to lymphocytes in peripheral blood is associated with overall survival in a large cohort of noncancer patients. We propose NLR is not specific to disease-bearing hosts but rather a more generally applicable biomarker reflective of shifting immune competence associated with aging, which is independently associated with survival. The neutrophil–lymphocyte ratio is an accessible, economical biomarker and should be exploited further for health risk stratification. The exceptional value of NLR lay not only in its statement of relative immune status, but also in

the conceivable modification of elements of age-related immune function for prevention of disease.

Future perspective

The analysis presented here forms the basis of a subsequent study designed not only to look at this issue in greater detail, but also to address the question of clinical utility of NLR. With improved understanding of the complexities of the immune system, manipulation of immune cell subsets and selective inhibition of cytokines, chemokines and transcription factors will become potentially powerful preventive strategies aimed at neutralizing acute inflammatory pathways while enhancing protective pathways [19,20]. Integrative biomarker studies, through the use of big data, will add to our appreciation of the evolving immune status of populations, their subsets and ultimately individuals [21]. We anticipate specific targeting of immune cell subsets will be employed to mitigate the risk of disease development and age-related immune senescence [22–24].

Author contributions

Conception and design of the work: JL Davis and DG Coit. Analysis and interpretation of data: JL Davis, V Moutinho Jr, KS Panageas and DG Coit. Drafting and revising the work: JL Davis, V Moutinho Jr, KS Panageas and DG Coit. Final approval of the version to be published: JL Davis, V Moutinho Jr, KS Panageas and DG Coit. Agreement to be accountable for all aspects of the work: JL Davis, V Moutinho Jr, KS Panageas and DG Coit.

Transparency declaration

JL Davis affirms that this manuscript is an honest, accurate and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Financial & competing interests disclosure

This work was partially supported by the NIH Core Grant (P30CA008748) award. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct disclosure

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

- Neutrophil–lymphocyte ratio (NLR) is associated with outcome in patients with various cancer types.
- We hypothesized NLR is associated with survival in noncancer patients.
- A retrospective cohort study was performed in patients lacking a cancer diagnosis and evaluated with a complete blood count.
- Median used to define high and low NLR.
- High NLR was associated with increasing age.
- High NLR associated with worse survival, independent of age.
- NLR is an independent predictor of survival in noncancer patients.

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