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Blood neutrophil count and neutrophil-to-lymphocyte ratio predict disease progression and mortality in two independent systemic sclerosis cohorts

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Abstract

Objective: To assess the predictive significance of blood neutrophil count and the ratio between neutrophil and lymphocyte count (neutrophil-to-lymphocyte ratio; NLR) for disease severity and mortality in systemic sclerosis (SSc).

Methods: Neutrophil and lymphocyte counts were prospectively measured in the Genetics versus ENvironment in Scleroderma Outcomes Study (GENISOS) and Scleroderma Lung Study (SLS) II. Forced vital capacity (FVC) % predicted and modified Rodnan Skin Score (mRSS) were used as surrogate measures for disease severity. Longitudinal analyses were performed using generalized linear mixed models. Cox proportional hazards models evaluated the predictive significance of these cell counts for mortality.

Results: Of the 447 SSc patients in the GENISOS cohort at the time of analysis, 377 (84.3%) had available baseline blood neutrophil and lymphocyte counts. Higher baseline neutrophil count and NLR predicted lower serially obtained FVC% (b=−4.74, p=0.009, b=−2.68, p=0.028, respectively) and higher serially obtained mRSS (b=4.07, p<0.001, and b=2.32, p<0.001, respectively). Longitudinal neutrophil and NLR measurements also significantly correlated with

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lower concurrently obtained FVC%s and higher concurrently obtained mRSS. Baseline neutrophil count and NLR predicted increased risk of long-term mortality, even after adjustment for baseline demographic and clinical factors (HR=1.42, p=0.02; HR=1.48, p<0.001, respectively). The predictive significance of higher baseline neutrophil count and NLR for declining FVC% and increased long-term mortality was confirmed in the SLS II.

Conclusion: Higher blood neutrophil count and NLR are predictive of more severe disease course and increased mortality, indicating that these easily obtainable laboratory studies might be a reflection of pathological immune processes in SSc.

Systemic sclerosis (SSc; scleroderma) is a heterogenous multiorgan disease characterized by immune dysregulation, vasculopathy, and excessive extracellular matrix deposition leading to skin and internal organ fibrosis (1). SSc has the highest mortality amongst major rheumatic diseases (2), in large part due to our fragmented understanding of its disease pathogenesis. Furthermore, the disease course is highly variable but there are limited prognostic markers to inform clinical care and trial design. An expanded understanding of key mechanisms driving SSc and objective markers of disease progression are critical for the development of targeted treatments for this potentially devastating disease. Interstitial lung disease (SSc-ILD) is the leading cause of disease-related mortality (3). The course of SSc-ILD is highly variable, but there are no predictors of SSc-ILD progression widely utilized in clinical practice (4). Skin fibrosis leading to skin thickening and decreased mobility is another prominent manifestation of SSc (1). Rapid progression of skin thickness and diffuse disease (dcSSc) are independent predictors of mortality in SSc (5).

Contrary to rodents (6), neutrophils are the largest component of circulating leukocytes in humans (7). This restricts the use of murine models while underscoring the importance of direct human data in understanding the role of neutrophils in human diseases. Despite their critical role during the inflammatory response (7), neutrophils are under-researched in SSc. Presence of a neutrophil gene expression signature was first described in the baseline peripheral blood cell RNA samples of Scleroderma: Cyclophosphamide or Transplantation (SCOT) trial and subsequently replicated in an independent observational cohort (8). In the SCOT trial (9), a decline in the neutrophil gene expression signature correlated with an improvement in the forced vital capacity (FVC) % predicted (8). Moreover, the neutrophil gene expression signature in peripheral blood cell RNA samples showed a moderate correlation with the concurrent neutrophil count. In addition to an upregulation of the neutrophil transcript module, SSc patients showed a downregulation of cytotoxic/natural killer (NK) transcript modules (8). Interestingly, this “inverse” lymphocytic signature can exist independent of the activated neutrophil transcript module (8, see Supplementary Figure 2).

Building upon the above recent discoveries and capitalizing on prospectively collected immune cell counts and clinical data in two well-phenotyped SSc populations, we determined herein whether blood neutrophil count and/or the ratio between circulating neutrophils and lymphocytes (neutrophil-to-lymphocyte ratio; NLR) were predictive of more severe disease and worse mortality in SSc.

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PATIENTS AND METHODS

GENISOS cohort

The Genetics versus ENvironment in Scleroderma Outcomes Study (GENISOS) is a prospective, observational cohort study of patients with early SSc based at the University of Texas Health Science Center at Houston. All patients met the ACR/EULAR Classification Criteria for SSc (10). All patients have a disease onset less than 5 years from the first non-Raynaud's phenomenon symptom at enrollment. Patients are seen every six months for the first three years after the first visit, then annually. At the time of analysis, 447 patients were enrolled in the GENISOS cohort from which 377 participants had a baseline neutrophil and lymphocyte count available. Baseline and follow-up study visits from these participants, consisting of 2,169 time points were included in the present study. The median follow-up time for study visits was 3 years.

Blood leukocyte measurements

At each visit, a complete blood count was obtained in a commercial clinical laboratory as part of the study protocol, which contained the neutrophil and lymphocyte counts. NLR was calculated by taking the ratio of the absolute neutrophil count to lymphocyte count.

Clinical outcome measures

Skin thickness was assessed by modified Rodnan Skin Score (mRSS) at the baseline and follow-up visits by rheumatologists who had completed mRSS training sessions.

Pulmonary function tests (PFTs) were performed at the initial visit and annually thereafter. Serially obtained FVC, expressed as the percentage of predicted FVC (FVC%), was used as a surrogate measure for SSc-ILD. FVC% values were calculated according to the patient's age, height, weight, sex, and ethnicity using consistent reference values (11). All PFT data fulfilled the American Thoracic Society/European Respiratory Society criteria for pulmonary function testing (12) and were reviewed by two pulmonologists.

For the longitudinal analysis, of those patients with a baseline neutrophil and lymphocyte count, the number of serially obtained FVC and mRSS measurements were 1,367 and 2,011 respectively.

Survival data collection

The vital status of each patient was obtained through medical records and queries, obtained from patients' charts, the National Death Index (NDI) at the Centers for Disease Control and Prevention (13) and the Social Security Death Index (SSDI).

Independent validation cohort

The validation cohort was comprised of all patients enrolled from the Scleroderma Lung Study (SLS II) trial (NCT00883129) who had baseline neutrophil and lymphocyte count available. SLS II was a randomized, double-blind Phase II clinical trial conducted at 14 US medical centers comparing the safety and efficacy of 2 years of mycophenolate mofetil (MMF) or 1 year of oral cyclophosphamide (CYC) followed by 1 year of placebo for

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patients with SSc-ILD. Key inclusion criteria were the following: SSc with limited or diffuse cutaneous involvement; age 18-75 years; disease onset within 7 years; and clinically significant ILD attributable to SSc. Daily prednisone use was restricted to ≤ 10 mg. For complete inclusion and exclusion criteria, see original publication (14). The analysis of serially obtained FVC% focused on the 3- to 12-month measurements because both treatment arms were receiving active immunosuppressive treatment during that time period. The vital status after completion of the two-year study period was ascertained by contacting the patients or his/her previously designated contact person. If the patient or his/her previously designated contact person could not be reached, investigators searched publicly available death registries (e.g. NDI, SSDI and online obituaries) (15, see Supplementary Appendix). SLS II participants were followed for up to 8 years after the first patient had been randomized.

Statistical analysis

GENISOS cohort: Neutrophil count, lymphocyte count, and NLR were $\log_2$-transformed for these analyses in order to achieve more normal distributions. Log-transformed distributions for all three parameters are shown in Supplementary Figure 1. The association of baseline demographic and clinical factors with concurrently obtained neutrophil count, lymphocyte count and NLR were assessed by linear regression.

The longitudinal analysis was carried out using generalized linear mixed models. First the predictive significance of baseline neutrophil, lymphocyte, and NLR for the serially obtained mRSS and FVC% measurements was investigated. Next, the correlation of longitudinally obtained neutrophil, lymphocyte, and NLR counts as time-varying independent variables with the concurrently obtained serial mRSS and FVC% measurements (from the same visit) were calculated. The longitudinal models were also extended to include race and baseline glucocorticoid use (prednisone equivalent dose > 10 mg per day) as independent variables. Race adjustment was included because race is associated with differential blood counts in the general population (16). Adjustment for glucocorticoid use was included because glucocorticoids can increase neutrophil counts by demargination. All models included time as an independent variable and used random intercept, as well as random slope for time. This analytic approach accounts for within-individual correlation in outcome measures by random effects.

The date of enrollment into the study was used as the starting point for the mortality analysis. All-cause mortality was used for the analysis. The death search was conducted in May of 2017. First, univariable Cox proportional hazards regression was conducted to identify baseline demographic and clinical factors that were associated with mortality. All demographic and clinical variables achieving a 0.1 significance level in the univariable model were included in the subsequent multivariable models. Two approaches were pursued to examine the predictive significance of blood count variables for mortality in the multivariable model. First, only demographic variables reaching $p<0.1$ in the univariable model were included in addition to the baseline blood count variable. Subsequently, the model was extended to include clinical variables that reached $p<0.1$ which could potentially be in the causal chain mediating the effect of blood counts on the mortality.

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Receiver operating characteristic (ROC) curves were generated to summarize the ability of baseline log_2-transformed NLR values to predict worse survival at 3, 5, and 10-years after the baseline visit. The optimal cut-off for log_2NLR at 5 years was selected based on maximizing the Youden index (17).

SLS II study: The association of baseline, log-transformed neutrophil counts with the course of FVC% measured at 3-month increments from 3 to 12 months was examined by a joint model combining mixed effects model for the longitudinally obtained FVC%s with a survival model to account for nonignorable missing data. The same method was used for the primary clinical outcome analysis in SLS II (14) and published SLS II biomarker studies which included adjustment for baseline disease severity (i.e., baseline FVC%) and treatment arm assignment (18).

The association of baseline neutrophil count and NLR with long-term mortality was examined by a separate Cox proportional hazards regression model. This analysis was adjusted for baseline age and treatment assignment.

All tests were 2-sided. The joint analyses were performed using the R package JMbayes and all other analyses were conducted in STATA v.12 (StataCorp LLC, College Station, TX).

RESULTS

Overall, 377 out of 447 SSc patients within the GENISOS cohort had a baseline neutrophil and lymphocyte count available (84.3%). The baseline demographic and clinical characteristics of these patients were similar to all patients enrolled in the GENISOS cohort, as summarized in Table 1. Focusing on those patients with available baseline neutrophil and lymphocyte counts, the proportion of male patients was 17.8%. Fifty-nine percent of patients had diffuse cutaneous SSc and mean (SD) disease duration at baseline was 2.5 (1.5) years.

Higher neutrophil count and NLR were associated with more severe disease features at enrollment in the GENISOS cohort:

Baseline neutrophil count and NLR were associated with markers of disease severity (see Supplementary Table 1). Higher baseline neutrophil counts were associated with diffuse cutaneous disease ($p<0.001$), absence of anti-centromere antibodies ($p=0.002$), and male gender ($p=0.001$). Moreover, higher neutrophil counts were associated with higher baseline mRSS and lower FVC% ($p<0.001$ and $p=0.035$, respectively).

Baseline lymphocyte count was negatively associated with male gender and age at enrollment. Moreover, a higher baseline lymphocyte count was associated with presence of anti-topoisomerase antibodies ($p=0.014$). No significant associations between baseline lymphocyte count and mRSS or FVC% were observed.

Baseline NLR demonstrated associations similar to those of the neutrophil count with diffuse cutaneous involvement ($p=0.012$), absence of anti-centromere autoantibody ($p=0.002$), and male gender ($p<0.001$). Moreover, higher NLR was significantly associated with anti-RNA polymerase II antibody positivity ($p=0.013$). NLR was significantly

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associated with higher mRSS, but was not significantly associated with FVC% (p<0.001 and p=0.063, respectively).

Higher neutrophil count and NLR predicted lower forced vital capacity lung volume over time in the GENISOS cohort:

Higher baseline neutrophil count predicted lower serially obtained FVC%s over time (point estimate = −4.74, 95% CI [−8.29 to −1.20], p=0.009). Moreover, higher longitudinal neutrophil counts correlated with lower concurrently obtained serial FVC%s over time (point estimate= −1.79, 95% CI [−2.95 to −0.64], p=0.002).

Baseline lymphocyte count did not predict longitudinal FVC% measurements (p=0.523). However, higher serially obtained lymphocyte counts correlated with higher concurrently obtained FVC% measurements over time (point estimate=1.43, 95% CI [0.45 to 2.40], p=0.004).

As observed with neutrophil count, higher NLR at baseline predicted lower serially obtained FVC% (point estimate = −2.68, 95% CI [−5.06 to −0.31], p=0.028) and higher longitudinal NLR values correlated with lower concurrently obtained FVC% measures over time (point estimate = −1.52, 95% CI [−2.27 to −0.77], p<0.001). Baseline and serially measured NLR values both remained significant predictors of longitudinal FVC% decline after adjustment for race and baseline prednisone use (Supplementary Tables 2 and 3, respectively). The relationship between longitudinal $\log_2$NLR and predicted longitudinal FVC%s is demonstrated graphically in Supplementary Figure 2.

Higher neutrophil count and NLR were associated with more severe skin involvement in the GENISOS cohort:

Baseline neutrophil count was also predictive of more severe skin involvement, assessed by higher serially obtained mRSS measurements over time (point estimate = 4.07, 95% CI [2.47 to 5.67], p<0.001). As observed with FVC%, longitudinal neutrophil counts also correlated with worse skin involvement as assessed by higher concurrently obtained mRSS over time (point estimate = 1.53, 95% CI [0.53 to 1.72], p<0.001).

Neither baseline nor serially obtained lymphocyte counts predicted/correlated with skin involvement over time as assessed by longitudinal mRSS measurements (p=0.208 and p=0.099, respectively).

Higher baseline NLR predicted higher serially obtained mRSS measurements (point estimate = 2.32, 95% CI [1.24 to 3.40], p<0.001). Furthermore, longitudinal NLRs positively correlated with concurrently obtained mRSS measurements over time (point estimate = 0.73, 95% CI [0.34 to 1.12], p<0.001). Baseline and serially obtained NLR remained an independent predictor/correlate of longitudinal mRSS even after adjustment for race and baseline prednisone use (Supplementary Tables 2 and 3, respectively). The relationship between longitudinal $\log_2$NLR and predicted longitudinal mRSS is demonstrated graphically in Supplementary Figure 2.

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Higher neutrophil count and NLR were predictors of mortality in GENISOS cohort:

Higher baseline neutrophil count and NLR were predictive of higher mortality in the univariable models (p=0.002 and p<0.001, respectively), along with older age at enrollment, and African American race (Supplementary Table 4). Conversely higher baseline lymphocyte count (p=0.001) along with anti-centromere positivity and higher FVC% predicted lower mortality. ROC curves illustrated NLR to be a consistent predictor of mortality 3, 5, and 10 years after the first visit. Using the $\log_2$ NLR cut-off of 1.56 (corresponding to raw NLR=2.95), the sensitivity and specificity for this NLR threshold value as a mortality predictor at 5 years were 74% and 55%, respectively (Supplementary Figure 3).

Higher neutrophil count and NLR predicted mortality after adjustment for demographic features and clinical parameters in GENISOS cohort:

First multivariable models after adjustment for demographic variables (age, gender, and race), were examined. Higher baseline neutrophil count and NLR remained significant predictors of higher mortality (p<0.001 for both analyses) while higher baseline lymphocyte count predicted lower mortality (p=0.001) after adjustment for these demographic variables (Table 2).

Notably, higher neutrophil count and NLR also remained significant predictors of higher mortality after extension of the multivariable model with baseline clinical variables (anti-centromere positivity, FVC%, mRSS, and prednisone use) (p=0.017 and p=0.001, respectively) while higher lymphocyte count predicted lower mortality in this model (p=0.016; Table 3).

Predictive significance of neutrophil count and NLR was confirmed in the validation cohort:

Of the 142 patients enrolled in SLS II, 134 (94.4%) had available baseline neutrophil, lymphocyte, and NLR with a mean (SD) value of 5,602 (2,363), 1,670 (660), and 4.24 (3.47), respectively. Supplementary Table 5 shows the baseline demographic and clinical characteristics of SLS II patients with available baseline cell counts.

Similar to results of the GENISOS cohort, higher neutrophil count and NLR were predictive of worse disease course. Specifically, higher baseline neutrophil count and NLR predicted worse ILD course as captured by lower serially obtained FVC% within 3-12 months after adjustment for baseline disease severity and treatment arm (p=0.033 and p<0.001, respectively; Table 4). However, neither baseline neutrophil count nor NLR were predictive of mRSS during follow-up visits (data not shown).

Higher baseline lymphocyte counts predicted higher serially obtained FVC% within 3-12 months after adjustment for baseline disease severity and treatment arm (p=0.018; Table 4). Lymphocyte count was not predictive of mRSS progression (data not shown).

Notably, as observed in the GENISOS cohort, higher baseline neutrophil count predicted higher long-term mortality after adjustment for age and treatment arm (Hazard ratio=2.22, 95% CI [1.02 to 4.80], p=0.044; Table 5). However, the predictive significance of baseline

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lymphocyte count for mortality did not reach statistical significance (p=0.075). Higher baseline NLR also predicted increased mortality in SLS II (Hazard ratio=1.84, 95% CI [1.17 to 2.91], p=0.009; Table 5).

DISCUSSION

In the present study, baseline blood neutrophil counts and NLR predicted mortality, and long-term progression of both skin and pulmonary disease in early SSc patients enrolled in a prospective observational cohort. The capacity of both baseline neutrophil count and NLR to predict worsening lung disease and increased mortality was confirmed in SSc patients enrolled in the SLS II. Cumulatively, these results provide direct clinical support for a pathological role of neutrophils both independently and possibly via interaction with lymphocytes in SSc pathogenesis.

We previously reported upregulated peripheral blood neutrophil and down-regulated cytotoxic/NK module gene expression signatures in early SSc patients (8). The neutrophil signature showed a weak but statistically significant inverse correlation with the lymphoid lineage cytotoxic/NK cell module (rs=−0.25, p=0.047). While this inverse correlation was observed between the two modules, they could exist independent of one another, indicating that an imperfect overlap between an increased neutrophil signature and a decreased lymphocyte signature exists such that a ratio between the two (i.e., NLR) would be expected to provide a more comprehensive view of SSc molecular signatures than each independent signature alone. The observed predictive significance of NLR for disease severity in the present study supports the notion that enrichment of neutrophils and underrepresentation of lymphocytic cells are a reflection of the pathologic immune dysregulations observed in SSc peripheral blood cells (8). Further, these observations underscore the complex relationship between the innate and adaptive immune system in SSc.

Aberrant immune system activation plays a critical role in the development and progression of SSc. Historically, immune dysregulation in SSc has been explored with a focus on the adaptive immune system, which drives the production of autoantibodies by T helper cell-dependent activation by B cells. Recent evidence demonstrates a role for innate immune cells in autoimmune disease by functioning as antigen presenting cells and accessory cells that secrete soluble factors (19).

Neutrophils serve numerous critical functions during the early inflammatory response. Neutrophils phagocytose foreign material, produce reactive oxygen species (ROS), release specialized granules by a process known as degranulation, and communicate with other immune cells by secreting chemokines and cytokines (18, 19). Extracellular ROS activates the endothelium (22) and stimulates surrounding immune cells, amongst other functions (21). Neutrophil granules contain enzymes including matrix metallopeptidases (MMPs), elastase and cathepsins which can cleave extracellular matrix components, modulating tissue remodeling during fibrosis (23). Activated neutrophils are capable of producing neutrophil extracellular traps (NETs), which contain DNA and histones interlaced with modified proteins, by a process known as NETosis (24). Neutrophils crosstalk with numerous innate and adaptive immune cells during the inflammatory response. For instance, neutrophils drive

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T helper 17 cell activation (25), induce an interferon (IFN) response (26), and secrete known pro-fibrotic and pro-inflammatory mediators such as interleukin-6 and transforming growth factor β (19, 25). Given their diverse contributions to immune regulation, perturbations in neutrophil homeostasis would be expected to have widespread implications in complex autoimmune inflammatory conditions, such as SSc.

It is well supported that overabundant dysfunctional neutrophils play a critical role in other autoinflammatory and fibrotic diseases. Patients with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and other select rheumatic diseases have an excess of circulating neutrophils (28-31). Elevated neutrophil count in SLE patients is associated with neutrophil abnormalities and activation markers (31). Interestingly, low lymphocyte counts are associated with type I IFN activity (31), suggesting convergence of neutrophil and lymphocyte perturbations on IFN pathway regulation. Of note, SSc patients display a prominent type I IFN gene expression signature (32).

Perhaps the most well-supported role of neutrophils in autoimmune phenomena is through the production of NETs. NETs are well known sources of autoantigens in RA and SLE. RA patients produce autoantibodies against NET-derived citrullinated peptides which is considered a key early pathogenic event (33). In SLE, NETs contain MMPs which not only act as self-antigens but also activate the vascular endothelium (34). NETs can also induce other immunomodulatory factors, notably type I IFNs (26). NETs may also play a role in the pathogenesis of fibrosis. NETs can induce activation and differentiation of lung fibroblasts *in vitro* (35). Further, NETs derived from SLE patients enhance the fibrotic phenotype of cultured skin fibroblasts (36).

Elevated neutrophil count has been reported in SSc patients (26, 35). However, the role of neutrophils in SSc is for the most part, unknown in the context of the larger disease pathology. Studies suggest numerous potential roles of neutrophils in specific SSc phenomena. In SSc, damage to vascular endothelial cells recruits neutrophils via a variety of chemotactic agents. Neutrophils also promote fibrogenesis indirectly through cross-talk with other immune cells such as macrophages (21, 28). SSc neutrophils appear to be primed for reactive oxygen species (ROS) generation and respond to activated endothelium in SSc. This local production of ROS may propagate endothelial dysfunction mediating fibrosis in SSc (38). Various ROS agents have been shown to be associated with a diffuse SSc with ILD phenotype (39). Neutrophils may also be a source of autoantigens in SSc. Isolated neutrophils from SSc patient blood have an increased production of NETs compared to neutrophils from healthy controls (40). Platelet-derived microparticles from SSc patients can activate neutrophils and induce production of neutrophil extracellular traps (41). Studies to thoroughly elucidate the mechanisms by which dysregulated neutrophils contribute to the onset and progression of SSc are limited. The findings reported here provide direct clinical premise upon which to support further investigations into the pathologic role of neutrophils in SSc.

Blood neutrophil profiling has previously been shown to provide insight into disease activity in rheumatic disease. Serum markers of neutrophil activity, both individually and as a composite panel predict early erosive disease and extraarticular involvement (42), and

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response to therapy in RA (43). While blood neutrophil count is used as an independent predictor of mortality in non-autoimmune pathologies such as cardiovascular disease (44) and end stage renal disease (45), to our knowledge this is the first report demonstrating blood neutrophil count as an independent predictor of mortality in SSc.

Although blood neutrophil count clearly has clinical relevance, the evidence supporting NLR as a more comprehensive indicator of disease status and mortality risk is even more robust. In other systemic autoimmune disorders, NLR has been recognized as being reflective of disease activity (46), predictive of treatment response (47), and associated with increased overall mortality (48). In SSc where NLR is elevated, limited but promising studies have proposed NLR as a useful clinical metric. Baseline NLR correlated with radiographic evidence of ILD among female SSc patients in a cross-sectional analysis (49). In a retrospective study of SSc patients with ILD, higher NLR correlated with worse lung function at baseline (50). Recently, NLR was shown to positively correlate with disease activity scores, mRSS, and CRP at baseline (37). In combination with previous cross-sectional reports, the longitudinal findings presented here support NLR as a clinical predictor of disease severity and mortality.

This study has several strengths. Overall limitations of previous investigations into NLR and neutrophil counts include cross-sectional designs, low number of patients, and a lack of longitudinal data and survival analysis. Our report addresses these limitations. GENISOS is a large well-characterized, multi-ethnic cohort of early SSc patients, facilitating robust investigation of clinical and biological data. The long-term, longitudinal data from the GENISOS cohort allowed for sufficiently powered analyses of serially obtained clinical outcome measures. Further, key findings were validated in a multicenter, clinical trial cohort of patients with SSc-ILD. GENISOS and SLS II patients had distinct geographic and clinical differences, increasing the generalizability of our study results.

Potential weaknesses include enrichment of the GENISOS cohort for patients with diffuse cutaneous disease due to referral bias to a tertiary referral center. Moreover, treatment regimens were heterogenous in the GENISOS study. While we accounted for glucocorticoid use, we did not account for immunosuppression due to concerns regarding confounding by indication bias. However, the predictive significance of neutrophil count and NLR for survival and ILD progression was replicated in the SLS II cohort in which immunosuppression was standardized and homogenous.

Taken together, our findings along with the recent discovery of a peripheral blood neutrophil gene expression signature support an important role for neutrophils in SSc, which merits further investigation. Mechanistic studies further investigating the modulatory role of the neutrophils and its relationship to adaptive immunity can provide important insights into pathogenesis of SSc and its complications. This study also demonstrates the potential role of peripheral blood neutrophil count and NLR as markers for disease progression in SSc.

Supplementary Material

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Significance and Innovations

- Higher neutrophil count and neutrophil-to-lymphocyte ratio predict lower forced vital capacity over time in patients with systemic sclerosis.
- Higher neutrophil count and neutrophil-to-lymphocyte ratio predict increased long-term mortality in systemic sclerosis.
- Paralleling findings at the gene expression level, a higher peripheral blood neutrophil and lower lymphocyte counts might be a reflection of pathological immune processes in systemic sclerosis, and a marker for more severe disease.

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Table 1.

Baseline characteristics of SSc patients in GENISOS cohort [*]

Characteristic	All patients (n = 447)	Patients with available baseline neutrophil and lymphocyte counts (n=377)	Number of patients with available data
Male gender	77 (17.2)	67 (17.8)	377
Age at enrollment, mean ± SD years	48.3 ±13.3	48.4 ±13.3	377
Race			377
White	220 (49.2)	187 (49.6)	
African American	83 (18.6)	69 (18.3)	
Latino	124 (27.7)	104 (27.6)	
Other	20 (4.5)	17 (4.51)	
Diffuse cutaneous involvement	267 (59.9)	223 (59.2)	377
Disease duration, mean ± SD years [†]	2.5 ±1.5	2.47 ±1.50	377
Anti-centromere antibody	60 (14.1)	46 (12.6)	364
Anti-topoisomerase I antibody	81 (18.7)	69 (18.6)	372
Anti-RNA polymerase III antibody	94 (21.9)	85 (23.0)	369
Deceased	138 (30.9)	122 (32.4)	377
Baseline mRSS, mean ± SD	16.5 ±11.8	16.2 ±11.7	367
Baseline FVC%, mean ± SD	82.8 ±21.5	82.5 ±21.7	329
Baseline prednisone use [ϕ]		24 (6.4)	373
Baseline immunosuppression [‡]		97 (25.8)	376
Methotrexate		46 (12.2)	
Mycophenolate mofetil		32 (8.5)	
Cyclophosphamide		10 (2.7)	
Azathioprine		9 (2.4)	
Other [§]		7 (1.9)	
Serum neutrophil count, mean ± SD [#]		5,179 ±2,403	377
Serum lymphocyte count, mean ± SD [#]		1,677 ±854	377
Serum neutrophil-to-lymphocyte ratio, mean ± SD		3.93 ±3.18	377

[*] GENISOS = Genetics Versus Environment In Scleroderma Outcome Study. Values are the number (percentage) unless indicated otherwise.

[†] Calculated from the onset of the first non–Raynaud's phenomenon symptom

[ϕ] Prednisone dose greater than 10 mg per day

[‡] Immunosuppressants = methotrexate, mycophenolate mofetil, cyclophosphamide, and azathioprine.

[§] Seven participants were on more than one immunosuppressive agent.

[#] Reported as cells/μL

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Table 2.

Baseline neutrophil count, lymphocyte count, and neutrophil-to-lymphocyte ratio as predictors of mortality after adjustment for baseline demographic covariables in GENISOS cohort

Characteristic	Neutrophil		Lymphocyte		NLR*	
	HR [95% CI]	P-value	HR [95% CI]	P-value	HR [95% CI]	P-value
Baseline count or ratio	1.67 [1.26, 2.22]	<0.001	0.66 [0.51, 0.85]	0.001	1.63 [1.34, 1.98]	<0.001
Male gender	1.41 [0.90, 2.21]	0.134	1.46 [0.93, 2.29]	0.097	1.20 [0.75, 1.90]	0.445
Age at enrollment	1.03 [1.02, 1.05]	<0.001	1.03 [1.01, 1.04]	0.001	1.03 [1.01, 1.04]	<0.001
Race		<0.001		<0.001		<0.001
African American†	2.56 [1.64, 4.00]	<0.001	2.57 [1.64, 4.03]	<0.001	2.84 [1.81, 4.46]	<0.001
Latino†	1.68 [1.09, 2.58]	0.018	1.70 [1.10, 2.62]	0.016	1.77 [1.15, 2.73]	0.010
Other†	0.23 [0.03, 1.66]	0.145	0.25 [0.04, 1.85]	0.176	0.24 [0.03, 1.70]	0.152

Analysis by multivariable Cox proportional hazards model

*Neutrophil-to-lymphocyte ratio

†Compared to White patients

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Table 3.

Baseline neutrophil count, lymphocyte count, and neutrophil-to-lymphocyte ratio as predictors of mortality after adjustment for baseline and clinical covariables in GENISOS cohort

	Neutrophil		Lymphocyte		NLR*	
Characteristic	**HR [95% CI]**	**P-value**	**HR [95% CI]**	**P-value**	**HR [95% CI]**	**P-value**
Baseline count or ratio	1.47 [1.07, 2.03]	0.017	0.69 [0.50, 0.93]	0.016	1.48 [1.18, 1.84]	0.001
Male gender	1.14 [0.69, 1.89]	0.604	1.15 [0.69, 1.90]	0.595	1.03 [0.62, 1.73]	0.902
Age at enrollment	1.03 [1.01, 1.05]	<0.001	1.03 [1.01, 1.05]	0.001	1.03 [1.01, 1.05]	0.001
Race		0.016		0.027		0.010
African American†	1.83 [1.10, 3.05]	0.021	1.71 [1.03, 2.84]	0.039	1.97 [1.18, 3.30]	0.009
Latino†	1.49 [0.89, 2.51]	0.133	1.45 [0.86, 2.43]	0.165	1.48 [0.88, 2.50]	0.137
Other†	0.16 [0.02, 1.19]	0.074	0.16 [0.02, 1.19]	0.074	0.17 [0.02, 1.23]	0.079
Anti-centromere antibody	0.94 [0.42, 2.10]	0.875	0.95 [0.42, 2.14]	0.910	1.01 [0.45, 2.28]	0.979
Baseline mRSS	1.00 [0.98, 1.02]	0.846	1.00 [0.99, 1.02]	0.692	1.00 [0.98, 1.02]	0.908
Baseline FVC%	0.97 [0.96, 0.98]	<0.001	0.97 [0.96, 0.98]	<0.001	0.97 [0.96, 0.98]	<0.001
Baseline prednisone use℘	1.27 [0.61, 2.62]	0.521	1.35 [0.66, 2.77]	0.416	1.17 [0.56, 2.43]	0.677

Analysis by multivariable Cox proportional hazards model

*Neutrophil-to-lymphocyte ratio

†Compared to White patients

℘Prednisone dose greater than 10 mg per day

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Table 4.

Baseline neutrophil count, lymphocyte count, and neutrophil-to-lymphocyte ratio as predictors of worsening SSc-ILD in SLS II

Characteristic	Neutrophil		Lymphocyte		NLR*	
	β [95% CI]	P-value	β [95% CI]	P-value	β [95% CI]	P-value
Baseline count or ratio	−1.14 [−1.18, −1.10]	0.033	1.34 [1.31, 1.38]	0.018	−1.16 [−1.19, −1.13]	<0.001
Baseline FVC%	0.91 [0.91, 0.91]	<0.001	0.90 [0.90, 0.90]	<0.001	0.90 [0.90, 0.91]	<0.001
Time variable	0.17 [0.16, 0.17]	0.024	0.17 [0.16, 0.18]	0.028	0.17 [0.17, 0.18]	0.014
Treatment arm†	0.99 [0.94, 1.04]	0.112	0.94 [0.89, 0.99]	0.139	1.04 [1.00, 1.08]	0.074

Analysis by joint model (14)

*Neutrophil-to-lymphocyte ratio

†Mycophenolate compared to cyclophosphamide treatment groups

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Table 5.

Mortality risk in SLS II patients as a function of baseline neutrophil count, lymphocyte count, and neutrophil-to-lymphocyte ratio

Characteristic	Neutrophil		Lymphocyte		NLR*	
	HR [95% CI]	P-value	HR [95% CI]	P-value	HR [95% CI]	P-value
Baseline count or ratio	2.22 [1.02, 4.80]	0.044	0.50 [0.23, 1.07]	0.075	1.84 [1.17, 2.91]	0.009
Age at enrollment	1.08 [1.03, 1.14]	0.001	1.09 [1.03, 1.14]	0.001	1.08 [1.03, 1.13]	0.002
Treatment arm†	0.94 [0.41, 2.14]	0.880	0.85 [0.36, 2.01]	0.719	0.82 [0.35, 1.91]	0.646

Analysis by multivariable Cox proportional hazards model

*Neutrophil-to-lymphocyte ratio

†Mycophenolate compared to cyclophosphamide treatment groups