Journal of the American Heart Association

ORIGINAL RESEARCH

Decreased Absolute Lymphocyte Count and Increased Neutrophil/Lymphocyte Ratio With Immune Checkpoint Inhibitor–Associated Myocarditis

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BACKGROUND: Myocarditis attributable to immune checkpoint inhibitor (ICI) therapy is a potentially fatal immune-related adverse event. Limited data have suggested an association between baseline and on-treatment absolute lymphocyte count (ALC) and neutrophil/lymphocyte ratio (NLR) and the development of other immune-related adverse events; there are no data characterizing the role of ALC and NLR in ICI-associated myocarditis.

METHODS AND RESULTS: This was a case control study of 55 patients with ICI myocarditis and 55 controls without any post-ICI immune-related adverse events. We leveraged clinical testing, where patients underwent routine serial blood counts before and with each ICI cycle to compare the baseline and change in ALC and NLR between cases and controls. The association between the change in these parameters with clinical variables and major adverse cardiac events was also tested. In cases, there was a statistically significant decrease in ALC with myocarditis from baseline (1.6 thousands per cubic milliliter (K/μL); interquartile range, 1.1–1.9 K/μL) to admission (1.1 K/μL; interquartile range, 0.7–1.3 K/μL; $P<0.001$). Similarly, there was an increase in NLR from baseline (3.5; interquartile range, 2.3–5.4) to admission (6.6; interquartile range, 4.5–14.1; $P<0.001$). There was no statistically significant change in controls. In follow-up, there were 20 events; larger decreases in ALC (44.6% versus 18.2%; $P<0.001$) or increases in NLR (156.5% versus 65.1%; $P=0.019$) were associated with major adverse cardiac events.

CONCLUSIONS: A reduction in ALC and an increase in NLR was seen with ICI myocarditis. A greater decrease in ALC or increase in NLR was associated with subsequent major adverse cardiac events.

Key Words: immune checkpoint inhibitor ■ lymphocyte ■ myocarditis ■ oncology

Immune checkpoint inhibitors (ICIs) are a revolutionary cancer therapy being increasingly applied to a broader range of cancers.[1,2] However, ICIs may stimulate T-cell activity against host tissues, resulting in immune-related adverse events (irAEs).[3] Myocarditis is an uncommon irAE associated with ICIs, with incidence rates varying widely from 0.1% to 2%.[4,5] This wide range may result from the difficulty of diagnosing myocarditis.[6] The number of cases of ICI-associated myocarditis is expected to increase with the rapid expansion of indications for an ICI.[7] Therefore, there is a need to improve our understanding of, and diagnostic strategies for, ICI-associated myocarditis. Typically, ICI-associated myocarditis occurs within the first few cycles of ICI treatment,[8,9] and this early presentation supports the testing of rational detection strategies.[10,11]

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Supplementary Material for this article is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.120.018306

For Sources of Funding and Disclosures, see page 11.

JAHA is available at: www.ahajournals.org/journal/jaha

CLINICAL PERSPECTIVE

What Is New?

- A reduction in absolute lymphocyte count and an increase in neutrophil/lymphocyte ratio was seen with immune checkpoint inhibitor myocarditis.

What Are the Clinical Implications?

- Measurement of absolute lymphocyte count and neutrophil/lymphocyte ratio could be part of a diagnostic algorithm and risk stratification in immune checkpoint inhibitor–associated myocarditis.
- These indexes could be applied when there is a clinical suspicion of myocarditis and when standard cardiac measures, such as ECG or troponin, are unavailable or indeterminate.
- Advantages of measuring absolute lymphocyte count and neutrophil/lymphocyte ratio are that they are both readily available, are standardized, are inexpensive, and are easily interpretable.

Nonstandard Abbreviations and Acronyms

ALC	absolute lymphocyte count
ICI	immune checkpoint inhibitor
irAE	immune-related adverse event
K/μL	thousands per cubic milliliter
NLR	neutrophil/lymphocyte ratio
MACE	major adverse cardiac event

Recent data suggest that both baseline and on-treatment changes in absolute lymphocyte count (ALC) and neutrophil/lymphocyte ratio (NLR) are associated with noncardiac irAEs. Specifically, data suggest that patients with higher baseline ALC and a lower baseline NLR have a greater risk for overall noncardiac irAEs.[12,13] Similarly, a decrease in ALC from baseline and an increase in NLR after ICI administration were associated with an increased risk of lung, gastrointestinal, and skin-related irAEs.[13,14] There are additional data to support the testing of these standard hematological parameters in ICI myocarditis. For example, an increased NLR has been reported in patients with heart failure and can predict poor prognosis,[15] and in patients with non-ICI myocarditis, an elevated NLR was associated with the extent of myocardial damage.[16] Therefore, the first goal of this study was to test the utility of ALC and NLR in the diagnosis of ICI myocarditis.

Beyond the diagnosis of myocarditis, there is a need for improved risk stratification of ICI myocarditis. The reported case fatality rate of ICI myocarditis is consistently high, at between 20 and 50%,[9,10] and high-dose steroids are usually prescribed once the diagnosis is made.[17,18] Previous data suggest that high-dose steroids may impact cancer outcomes and that there is need for biomarkers to guide treatment after the diagnosis with an irAE.[19] Therefore, a secondary aim was to test the association between baseline and on-treatment ALC and NLR with subsequent major adverse cardiac events (MACEs).

METHODS

The data, analytic methods, and study materials will be made available from the corresponding author on reasonable request after institutional approval and following institutional process.

Study Design

This was a retrospective case control study with 55 patients with ICI-associated myocarditis and 55 randomly selected controls without any irAEs post-ICI. Data from myocarditis cases were obtained from 2 centers in a single integrated network (Brigham and Women's Hospital and Massachusetts General Hospital, Boston, MA). Cases included in our analyses presented with ICI-associated myocarditis from November 2013 to April 2019. A total of 47% of ICI-associated myocarditis cases also had other irAEs. Controls were randomly selected from the same network in 1:1 ratio. Control patients were started on ICI during the same time frame as cases and were confirmed by independent chart review by an immuno-oncologist to not have developed any irAE (L.Z.). The institutional review board at each center approved the study, and the requirement for written informed consent was waived. The investigation conforms with the principles outlined in the Declaration of Helsinki.[20]

Covariates of Interest

Data on the covariates were extracted retrospectively from electronic medical records. Patient demographics, cardiovascular risk factors, medications, and cardiac biomarker levels were retrospectively extracted from electronic medical records. In addition, cancer-specific covariates, including cancer type, type of ICI, prior cardiotoxic chemotherapy, and radiation therapy, were recorded. Myocarditis-specific covariates included clinical presentation, physical examination, and admission cardiac biomarkers. We leveraged clinical testing where patients underwent routine serial complete blood cell count and differential counts before and with each ICI dose. In the myocarditis group, we recorded hematological parameters at 3 time points: baseline (ie, immediately before starting ICI), before

last ICI dose (ie, before clinical presentation with myocarditis), and at admission to the emergency room with myocarditis. Among the controls, we recorded these parameters at 2 time points: at baseline before starting ICI and before the last ICI dose. In the myocarditis group, standard clinical variables were also collected at the time of admission with myocarditis.

Definitions and Outcomes of Interest

Myocarditis was diagnosed either using clinical findings, biomarkers, and imaging features from the 2013 European Society of Cardiology guidelines[21] or from standard features present on pathological results. The main outcome of interest was the occurrence of MACEs. In previous investigations of patients on ICIs, MACE was defined as a composite of cardiovascular death, cardiac arrest, cardiogenic shock, and hemodynamically significant complete heart block.[10,22,23] Board-certified cardiologists at a local site adjudicated MACEs, according to a prespecified protocol. Cases where cardiac arrest, cardiogenic shock, or complete heart block led to death were counted as a cardiac death. When a patient had multiple MACEs, the date of the earliest event was defined as the time to first MACE.

Statistical Analysis

Baseline characteristics are presented as continuous variables and summarized as either mean±SD or as a median and interquartile range (IQR). Categorical variables are summarized as percentages. Differences in categorical variables are assessed using either the χ^2 test or the Fisher exact test. The primary measure of interest in this study was the percentage change in ALC from baseline. The secondary measure of interest was the percentage change in NLR from baseline. The first test performed was the comparison of change in the ALC and NLR over time among cases and controls, applying a repeated measures ANOVA. If the primary comparison was significant, differences between each time point were compared on the basis of the repeated measures ANOVA test. Comparisons between cases and controls at baseline and before the last ICI were made using a Mann-Whitney *U* test. The percentage change was calculated as follows: % change=([difference from baseline/baseline value]*100). Prespecified subanalyses were also performed, including only the biopsy-proven myocarditis cases (again matched 1:1 to controls). The start point was defined as the start of ICI therapy for cases and controls. For the analysis on MACEs, start point was defined as the date of admission with myocarditis. Patients were censored at first MACE or at last follow-up.

Receiver-operating characteristic curve was applied to calculate the optimal cutoff values for the percentage change in ALC or NLR. The cutoff point was determined using the Youden index.[24] Kaplan-Meier curves and the log-rank test were used to quantify the relationship between percentage change in ALC and NLR and MACE-free survival. All statistical tests were 2 sided, and 5% was set as the level of significance. Statistical analysis was performed using SPSS version 25 (IBM Corporation, Armonk, NY).

RESULTS

Baseline demographics and clinical characteristics are summarized in Table 1. In comparison with controls, myocarditis cases were evenly matched in age, cardiovascular risk factors, and cancer characteristics (Table 1). The mean age of the study cohort was 67±15 years. The most common indication for ICI therapy was melanoma. Among the cases, PD-1 (programmed cell death protein-1) inhibitor therapy was the most commonly prescribed. The median number of ICI cycles received was lower among cases compared with controls (2 [IQR, 1-5] cycles versus 4 [IQR, 2-9] cycles; P=0.024). A complete description of ICI therapies in cases and controls is shown in Table 1. Baseline blood parameters can be seen in Table 2. The median time to onset of myocarditis from starting an ICI was 51 days (IQR, 29-155 days). Among cases, the chief presenting complaints were shortness of breath (60%) and chest pain (24%), while 2 patients had no symptoms. In addition, among cases, 78% had an abnormal ECG on admission, troponin levels were elevated in 91%, and B-type natriuretic peptide (BNP) levels were elevated in 64%. Of the cases, 53 had an echocardiogram at admission, the mean ejection fraction (EF) was 51±18%, and 38% had a reduced EF of <50%. Among the cases, 31 were diagnosed with a heart biopsy, based on standard pathological criteria. Of the remaining 24 cases, 13 had cardiac magnetic resonance imaging consistent with myocarditis. In 8 of the remaining 11 cases, diagnoses were made with the combination of an elevated troponin and clinical symptoms without evidence of coronary ischemia (invasive angiography was performed in 4, stress test in 3 cases, and computed tomography angiography in 1 patient). In the 3 remaining cases, diagnoses were made with the combination of an elevated troponin and clinical symptoms. All 3 patients had recently started an ICI therapy and were admitted with signs and symptoms consistent with heart failure and an elevated troponin. All 3 patients had a preserved EF and a new pericardial effusion on

Table 1. Basic Characteristics for the ICI-Associated Myocarditis and Control Groups

Characteristic	ICI-Associated Myocarditis Group (n=55)	Control Group (n=55)	*P* Value
Sex (men), n (%)	41 (74.5)	30 (54.5)	0.046
Age, mean (SD), y	67 (15)	66 (16)	0.69
BMI, mean (SD), kg/m^2	26.4 (5.1)	25.8(6.5)	0.62
Ethnicity, n (%)			0.37
Non-Hispanic	51 (92.7)	51 (92.7)	
Hispanic	3 (5.5)	1 (1.8)	
Unknown	1 (1.8)	3 (5.5)	
Race, n (%)			0.95
White	52 (94.5)	51 (92.7)	
Black	1 (1.8)	1 (1.8)	
Asian	1 (1.8)	1 (1.8)	
Unknown	1 (1.8)	2 (3.6)	
Comorbidities, n (%)			
Diabetes mellitus	12 (22.2)	18 (33.3)	0.28
Hypertension	35 (63.6)	33 (61.1)	0.85
History of smoking	37 (67.3)	31 (57.4)	0.43
Coronary artery disease	9 (16.4)	7 (13.0)	0.79
Stroke or TIA	3 (5.5)	7 (13.0)	0.20
Heart failure	6 (10.9)	3 (5.6)	0.49
Atrial fibrillation	7 (12.7)	9 (16.7)	0.60
COPD	15 (27.3)	7 (13.0)	0.094
Cancer type, n (%)			0.92
Melanoma	17 (30.9)	19 (34.5)	
Lung	15 (27.3)	16 (29.1)	
Renal cell carcinoma	5 (9.1)	3 (5.5)	
Head and neck	3 (5.5)	4 (7.3)	
Other	15 (27.3)	13 (23.6)	
ICI type (most recent), n (%)			
CTLA-4	1 (1.8)	5 (9.1)	0.21
PD1	42 (76.4)	47 (85.5)	0.33
PDL1	3 (5.5)	0	0.24
CTLA4+PD1	7 (12.7)	3 (5.5)	0.20
CTLA4+PDL1	1 (1.8)	0	
Prior chemotherapy or radiation, n (%)			
None	25 (45.5)	8 (14.5)	0.001
Any	30 (54.5)	47 (85.5)	0.001
Anthracycline	1 (1.8)	1 (1.8)	1.000
Radiation	20 (36.4)	28 (50.9)	0.18
VEGF inhibitor	1 (1.8)	4 (7.3)	0.36
Tyrosine kinase inhibitor	1 (1.8)	5 (9.1)	0.21
MEK inhibitor	2 (3.6)	2 (3.6)	1.000
B-Raf inhibitor	2 (3.6)	1 (1.8)	1.000
Cardiovascular medications, n (%)			
Statin	25 (45.5)	16 (29.6)	0.11
Aspirin	21 (38.2)	15 (27.8)	0.31
β Blockers	17 (30.9)	16 (30.2)	1.00
ACEi or ARB	15 (27.3)	15 (27.3)	1.00

(*Continued*)

Table 1. Continued

Characteristic	ICI-Associated Myocarditis Group (n=55)	Control Group (n=55)	*P* Value
Calcium channel blocker	6 (10.9)	10 (18.5)	0.29
Loop diuretics	6 (10.9)	8 (14.8)	0.58
Thiazide diuretics	2 (3.6)	3 (5.6)	0.68
Aldosterone antagonists	2 (3.6)	2 (3.6)	1.00
Warfarin	3 (5.6)	3 (5.6)	1.00
Novel oral anticoagulants	2 (3.6)	2 (3.6)	1.00
LMWH	2 (3.6)	4 (7.4)	0.44

ACEi indicates angiotensin-converting-enzyme inhibitor; ARB, angiotensin II receptor blocker; BMI, body mass index; B-Raf, proto-oncogene B-Raf; COPD, chronic obstructive pulmonary disease; CTLA4, cytotoxic T-lymphocyte associated protein 4; ICI, immune checkpoint inhibitor; LMWH, low-molecular-weight heparin; MEK, mitogen-activated protein kinase kinase; PD1, programmed cell death protein-1; PDL1, programmed death ligand-1; TIA, transient ischemic attack; and VEGF, vascular endothelial growth factor.

transthoracic echocardiogram. Tumoral origin of the pericardial fluid was ruled out; therefore, they were treated as ICI myocarditis.

Absolute Lymphocyte Count

Among patients who developed ICI-associated myocarditis, the ALC at baseline was higher than in controls (1.6 thousands per cubic milliliter (K/μL) [IQR, 1.1–1.9 K/μL] versus 1.3 K/μL [IQR, 0.7–1.7 K/μL]; *P*=0.02). In cases, there was a statistically significant decrease in the ALC from baseline, to before the last ICI dose, to presentation with myocarditis (1.6 K/μL [IQR, 1.1–1.9 K/μL] to 1.4 K/μL [IQR, 0.9–1.6 K/μL] to 1.1 K/μL [IQR, 0.7–1.3 K/μL]; *P*<0.001) (Figure 1A). The decrease in ALC among cases occurred before the presentation with clinical myocarditis (*P*<0.001). In contrast, there was no change in the ALC over time in controls without any irAE (1.3 K/μL [IQR, 0.7–1.7 K/μL] to 1.5 K/μL [IQR, 0.9–1.9 K/μL]; *P*=0.12 (Figure 1A). Similar results were noted when the analysis was restricted to the biopsy-proven myocarditis cases and after exclusion of myocarditis cases who also had another irAE (Figure S1A and Figure S2A).

Neutrophil/Lymphocyte Ratio

Among patients who developed ICI-associated myocarditis, the NLR at baseline was not different compared with controls (3.5 [IQR, 2.3–5.4] versus 4.5 [IQR, 2.5–9.5]; *P*=0.11 (Figure 1B). Among ICI-associated myocarditis cases, there was an increase in the NLR from baseline, to before the last ICI dose, to presentation with myocarditis (3.5 [IQR, 2.3–5.4] to 4.1 [IQR, 2.3–6.4] to 6.6 [IQR, 4.4–14.1]; *P*<0.001) (Figure 1B). The increase in NLR among cases occurred before the presentation with clinical myocarditis (*P*=0.028; Figure 1B). In contrast, the NLR did not change over time in controls (4.5 [IQR,

Table 2. Baseline Blood Parameters in Patients With ICI-Associated Myocarditis and in Controls

Parameter	ICI-Associated Myocarditis Group (n=55)			Control Group (n=55)			*P* Value
	Median	IQR 1	IQR 3	Median	IQR 1	IQR 3	
White blood cell count, K/μL	7.81	6.23	9.10	7.14	5.62	8.55	0.25
Hemoglobin, g/dL	12.50	11.00	14.30	11.80	10.30	12.90	0.079
Hematocrit, %	37.90	34.00	43.20	36.00	31.10	38.70	0.073
Platelets, K/μL	220.00	189.00	288.00	237.00	169.00	305.00	0.63
Neutrophil count, %	70.00	64.00	75.30	72.80	64.20	81.60	0.34
Lymphocyte count, %	17.60	14.40	22.90	14.10	9.20	24.10	0.12
Monocyte count, %	9.10	7.40	11.30	8.20	6.60	11.80	0.12
Absolute neutrophil count, K/μL	5.19	4.01	6.73	4.86	3.60	6.72	0.53
Absolute lymphocyte count, K/μL	1.61	1.10	1.88	1.27	0.67	1.70	0.020
Absolute monocyte count, K/μL	0.68	0.53	0.89	0.63	0.43	0.78	0.083
Neutrophil/lymphocyte ratio	3.51	2.32	5.40	4.52	2.47	9.46	0.11

ICI indicates immune checkpoint inhibitor; IQR, interquartile range; and K/μL, thousands per cubic milliliter.

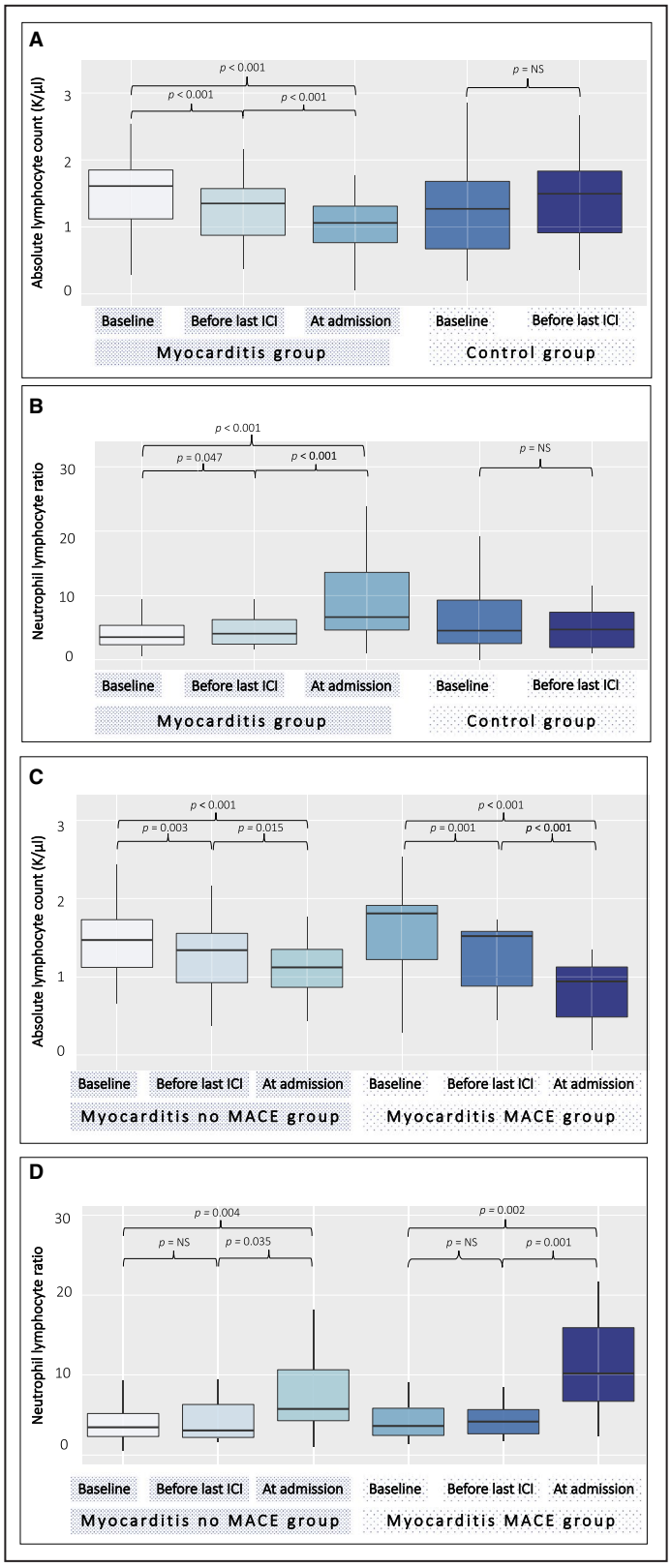

Figure 1. Bar plots of absolute lymphocyte count (ALC) and neutrophil/lymphocyte ratio (NLR) in patients with myocarditis and controls at different time points.

A, Bar plots of ALC in the myocarditis and control groups. **B**, Bar plots of NLR in the myocarditis and control groups. **C**, Bar plots of ALC in myocarditis cases with major adverse cardiac events (MACEs) and with no MACEs. **D**, Bar plots of NLR in myocarditis cases with MACEs and with no MACEs. ICI indicates immune checkpoint inhibitor.

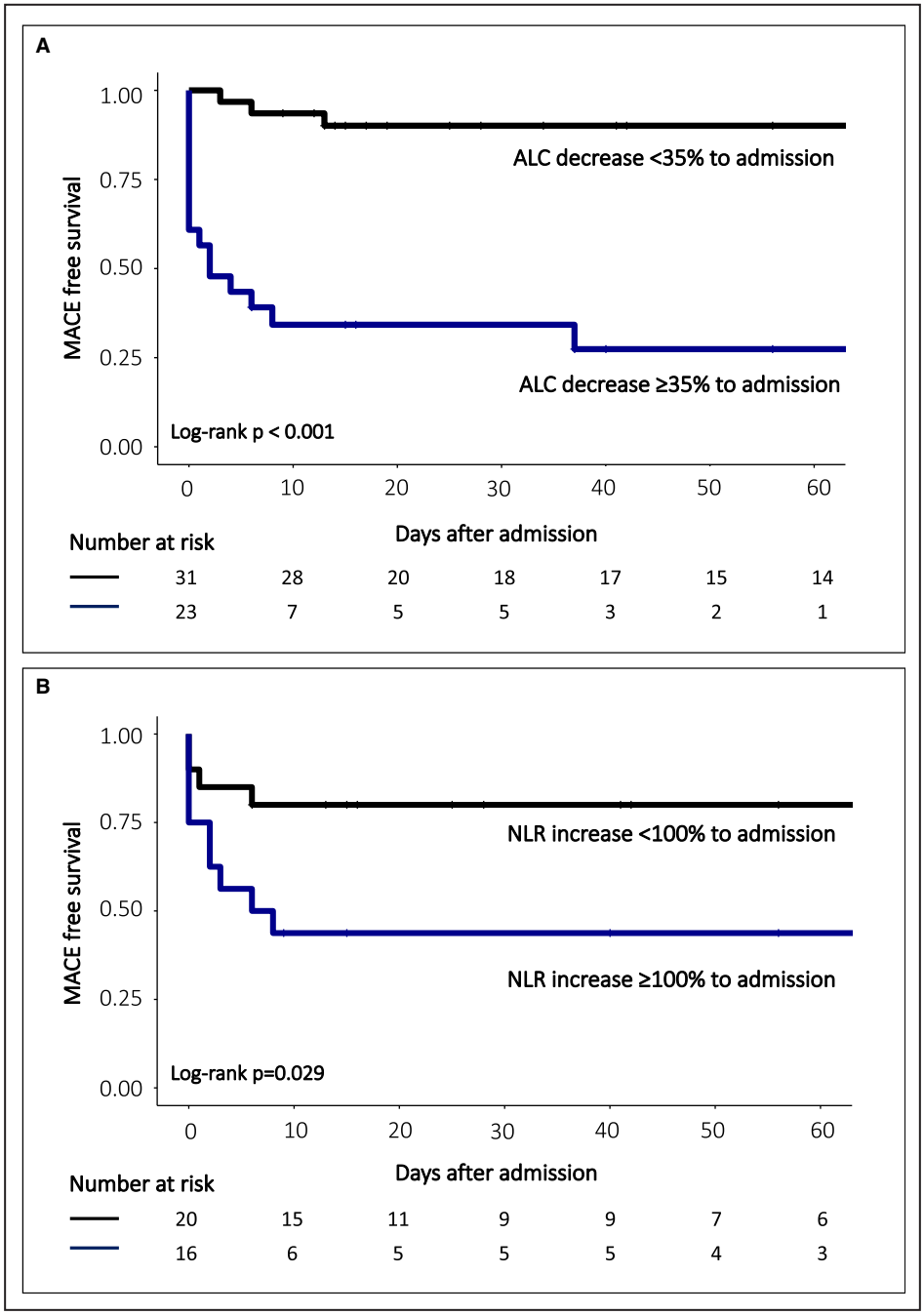

Figure 2. Receiver-operating characteristic (ROC) curve for determining the optimal cutoff points in the percentage change of absolute lymphocyte count (ALC) and neutrophil/lymphocyte ratio (NLR) for identifying patients with immune checkpoint inhibitor–associated myocarditis with subsequent major adverse cardiac events (MACEs).
A, The ROC curve applied for the percentage change in ALC in patients with myocarditis with subsequent MACEs vs patients with myocarditis without MACEs during follow-up. **B**, The ROC curve applied for the percentage change in NLR in patients with myocarditis with subsequent MACEs vs patients with myocarditis without MACEs during follow-up.

2.5–9.5] to 4.7 [IQR, 1.9–7.4]; *P*=0.78; Figure 1B). Similar results for the NLR were noted when the analysis was restricted to the biopsy-proven myocarditis cases and after exclusion of myocarditis cases who also had another irAE (Figure S1B and Figure S2B).

Association Between Percentage Change in ALC and NLR With Standard Clinical Variables

We tested the association of ALC and NLR with standard cardiac clinical variables, including blood

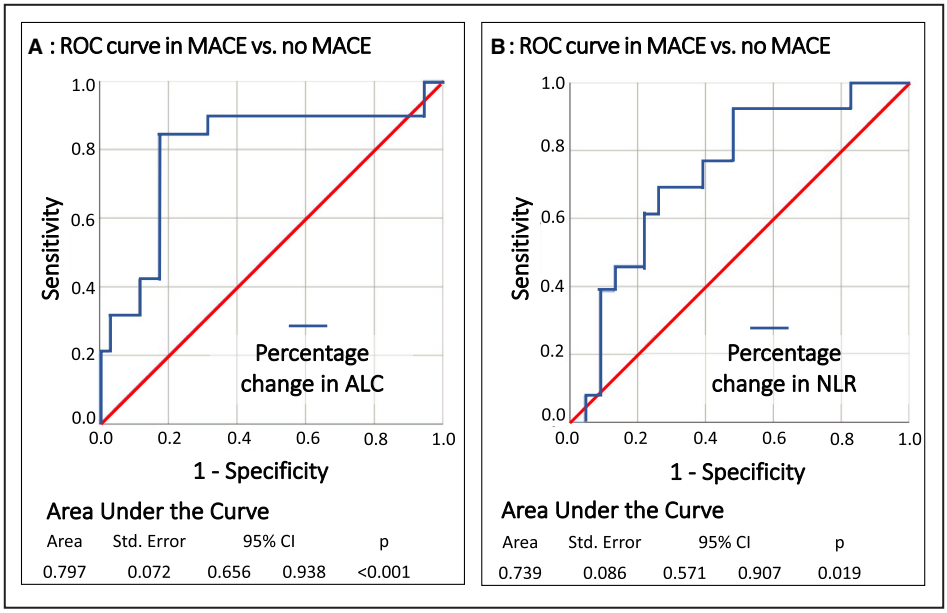

Figure 3. Kaplan-Meier curves based on previously defined cutoff values for major adverse cardiac event (MACE) free survival in patients with immune checkpoint inhibitor–associated myocarditis.
ALC indicates absolute lymphocyte count; NLR, neutrophil/lymphocyte ratio; ROC, receiver-operating characteristic; and Std., standard.

pressure and heart rate, ECG parameters, EF, and troponin and pro-BNP levels. The following parameters were significantly associated with ALC and NLR levels: heart rate, systolic blood pressure, and diastolic blood pressure. Specifically, a greater decline in ALC was associated with a higher heart rate at presentation (r=–0.42; P=0.011). Similarly, a larger increase in NLR correlated with a higher heart rate (r=0.36; P=0.03). There was a modest association between the change in ALC and lower systolic (r=0.32; P=0.02) and diastolic (r=0.35; P=0.009) blood pressures. No association was found between the ECG parameters at presentation, the EF, the troponin levels, the pro-BNP levels, and ALC or NLR levels. Similar results were noted for the correlation with heart rate when we included only the biopsy-proven myocarditis cases; however, correlation was not significant between ALC and blood pressure measures in the biopsy-proven case-control cohort.

Major Adverse Cardiac Events

Patients were followed up for a median of 41 days (IQR, 15–146 days) after admission with myocarditis. During follow-up, MACEs occurred in 20 cases (36.4%). The median time to MACE from admission with myocarditis was 1 day (IQR, 1–5 days). These events included complete heart block (n=8), cardiogenic shock (n=6), cardiac arrest (n=4), and 2 cardiovascular deaths. Among those with a subsequent MACE, there was a greater percentage decrease in ALC (from baseline to admission, 44.6% versus 18.2% [P<0.001]; and from before last ICI dose to admission, 24.3% versus 6.3% [P=0.013]; Figure 1C). Receiver-operating characteristic curves were applied to calculate the optimal cutoff point for MACE. A ≥35% decrease in ALC displayed the optimal combination of sensitivity (84%) and specificity (80%) for the association with MACE (area under the curve, 0.80; 95% CI, 0.66–0.94; P<0.001; Figure 2A). Using this cutoff value, a Kaplan-Meier curve was generated (Figure 3A). Similar results were found when we included only the biopsy-proven myocarditis cases and after exclusion of myocarditis cases who also had another irAE (Figure S1C and Figure S2C). The association between NLR and MACE was also tested. Among those with a subsequent MACE, there was a greater percentage increase in NLR (from baseline to admission, 156.5% versus 65.1% [P=0.019]; and from before last ICI dose to admission, 122.9% versus 19.7% [P=0.019]; Figure 1D). Receiver-operating characteristic curves were also applied to define the optimal cutoff value for the prediction of MACE. A ≥100% increase in NLR had a sensitivity of 69% and specificity of 70% in identifying those patients who subsequently developed MACEs (area under the curve, 0.74; 95% CI, 0.57–0.90; P=0.019; Figure 3B). Using this cutoff value, a Kaplan-Meier curve was also generated (Figure 3B), and again, similar results were found when we included only the biopsy-proven myocarditis cases and after exclusion of myocarditis cases who also had another irAE (Figure S1D and

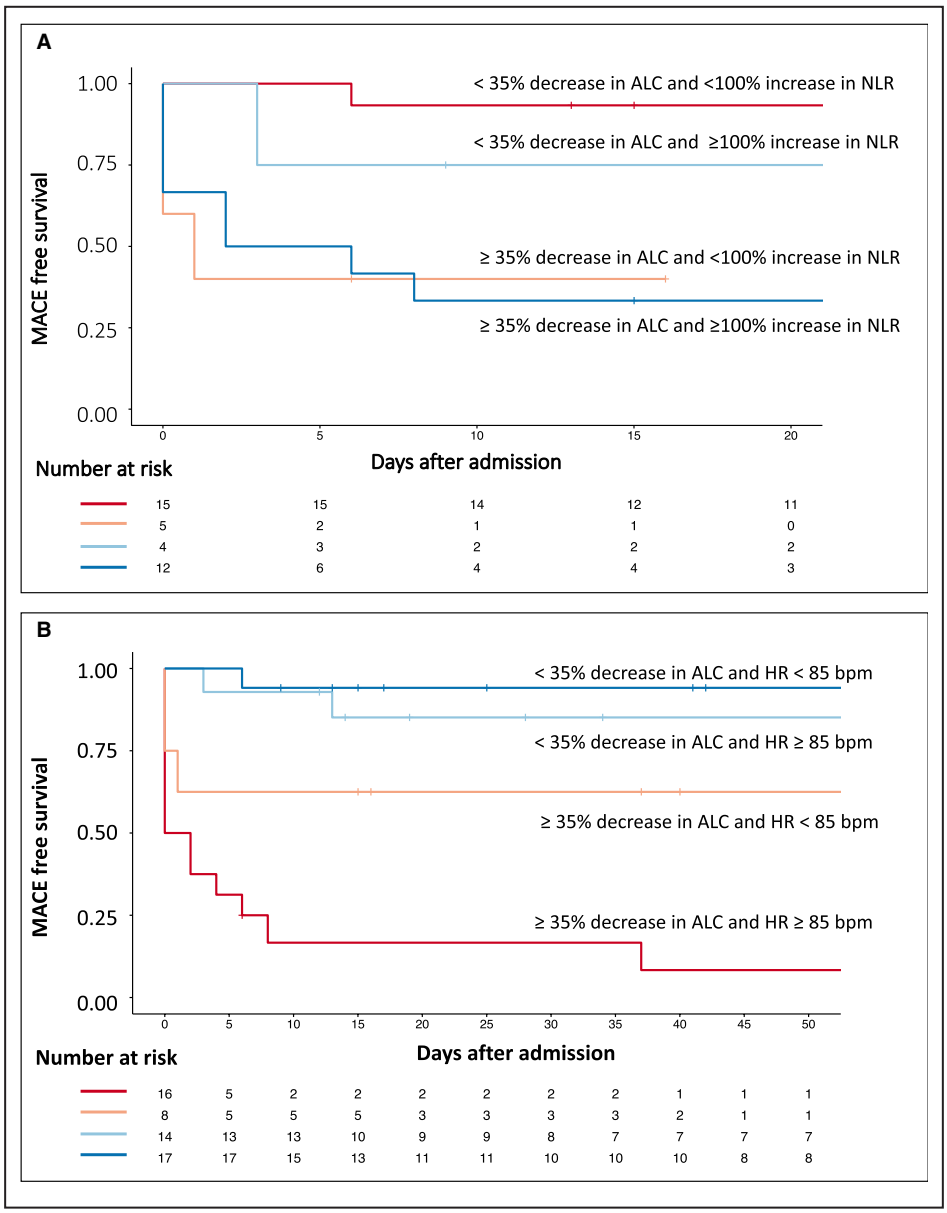

Figure 4. Kaplan-Meier curves based on previously defined cutoff values for major adverse cardiac event (MACE) free survival in patients with immune checkpoint inhibitor–associated myocarditis.
ALC indicates absolute lymphocyte count; bpm, beats per minute; HR, heart rate; and NLR, neutrophil/lymphocyte ratio.

Figure S2D). The combination of ≥35% decrease in ALC and ≥100% increase in NLR separated patients in MACE-free survival (Figure 4A). The combination of ≥35% decrease in ALC and heart rate ≥85 beats per minute at presentation separated patients in MACE-free survival (Figure 4B).

DISCUSSION

In this report, we compared the baseline and on-treatment changes in ALC and NLR among patients who developed ICI-associated myocarditis with controls who did not develop any irAE on ICI. There was a progressive statistically significant decrease in the ALC over time in patients who developed ICI-associated myocarditis and no statistically significant change in ALC among controls. Similarly, there was a progressive statistically significant increase in the NLR over time in cases and no statistically significant change in controls. There was an association between the magnitude of these changes and the clinical severity of the myocarditis, where a greater decrease in ALC,

and greater increase in NLR, correlated with a higher heart rate and a lower blood pressure. The magnitude of these changes was also associated with the occurrence of subsequent MACEs, where both the decrease in ALC and the increase in NLR were each predictive of adverse cardiac events.

There is a critical need to improve methods for detection of ICI-associated myocarditis. Surveillance is suggested for patients at risk for cardiac injury and for patients receiving or who have received standard cytotoxic (eg, doxorubicin) and targeted cancer therapies (eg, trastuzumab).[25] For example, among patients receiving trastuzumab, serial measurement of left ventricular EF is recommended.[25] However, serial measurement of left ventricular EF may not be useful among patients with ICI myocarditis as approximately 60% have a normal left ventricular EF.[10] A reduction in global longitudinal strain has been reported at presentation among patients with ICI myocarditis but no serial data exist testing whether the global longitudinal strain decreases before the development of clinical myocarditis.[23] Serum troponin and serial ECGs have also been proposed for detection of myocarditis.[5] These methods are inexpensive and widely available, and an increased troponin and abnormal ECG are common among patients with ICI myocarditis.[10] However, these approaches likely lack specificity in isolation,[10] and additional cost-effective and widely scalable approaches are needed. Patients being treated with ICIs undergo routine serial complete blood counts and differentials, from which the ALC and NLR are easily derived.

There are no prior data testing the role of ALC and NLR in the detection of myocarditis. However, there are significant data to provide a basis for this approach. In patients with advanced staged cancers, lymphopenia was proposed to be a surrogate marker of initial resistance to immunotherapy and a surrogate marker of several factors associated with advanced stage.[26] Increased NLR is associated with a reduced response rate to ICI therapy, and worse outcomes in many solid tumors among early and advanced stages were also reported.[27] In a retrospective review of 167 patients with cancer on ICI therapy, Diehl et al found that a higher baseline ALC was associated with an increased incidence of noncardiac irAEs.[12] In a study among patients with non–small-cell lung cancer, a low NLR at baseline was associated with the development of irAEs (odds ratio, 2.2; P=0.018) and the routine measurement of NLR was proposed.[13] In lung and gastrointestinal irAEs, the magnitude of the decrease in ALC was also associated with worse outcomes, where a ≥32% decrease in the lymphocyte count was associated with a 5-fold increased rate of irAEs.[14] There are also significant data on the role of ALC and NLR in different models of cardiac injury. In patients with cardiovascular diseases, lymphopenia has been shown to correlate with outcomes; NLR was a strong predictor of myocardial damage in both patients with acute myocardial infarction, who underwent percutaneous intervention,[28] and a small cohort of patients with myocarditis.[16] Before this report, there were no data characterizing the baseline and on-treatment changes in ALC or NLR among patients who develop ICI-associated myocarditis. Our study now provides novel data that ALC and NLR measurements could be used as part of a diagnostic algorithm in ICI-associated myocarditis. A decrease in ALC was not only observed at admission with ICI-associated myocarditis, but also before the last ICI dose. Surveillance of ALC and NLR may be an effective and rapid means of risk-stratifying and early screening ICI recipients to guide the selection of patients for additional, more specific, cardiovascular testing, such as cardiac magnetic resonance imaging; however, prospective trials will be needed to demonstrate the efficacy of this approach. These data suggest that measurement of these indexes could be applied when there is a clinical suspicion of myocarditis and when standard cardiac measures, such as ECG or troponin, are unavailable or indeterminate.

Beyond diagnosis of ICI myocarditis, there is also need for improved methods for risk stratification among patients with ICI myocarditis.[18] The use of combination ICI therapy has been described as a risk factor for ICI myocarditis,[10] and in our cohort, myocarditis cases received combination therapy in 14.5% versus 5.5% in controls; however, the difference was not significant. Consistent data have established that the morbidity and mortality with ICI myocarditis is high.[9,23] Cardiovascular mortality in ICI myocarditis ranges from 20% to 50%.[9,23] In contrast, the cardiovascular mortality rate with non-ICI myocarditis is far less than 5%.[29] High-dose corticosteroids are the first line of treatment for patients with ICI myocarditis,[17] but the use of high-dose corticosteroids may attenuate the anticancer efficacy of ICI therapy and adversely impact cancer outcomes.[19,30] An inexpensive and widely available biomarker that could stratify at the time of diagnosis the risk of subsequent adverse cardiac outcomes may be useful. However, early data using standard cardiac approaches have shown mixed outcomes.[5,23] For example, late gadolinium enhancement is common in non-ICI myocarditis, and it is a robust predictor of adverse events in non-ICI myocarditis.[31] In ICI myocarditis, late gadolinium enhancement was observed in <50% of patients with a preserved EF and, as a result, the presence of late gadolinium enhancement was not associated with adverse outcomes.[10,22] Herein, we present the first data in ICI myocarditis, where the magnitude of the decrease in ALC or increase in the NLR is associated with worse outcomes and correlated with heart rate and blood pressure at presentation. Advantages

of measuring ALC and NLR are, like troponin and measurement of global longitudinal strain, that they are both readily available, are standardized, are inexpensive, and are easily interpretable.

Limitations

Our findings should be interpreted within the context of the study design. This was a retrospective study where 24 of 55 myocarditis cases did not have biopsy-proven disease. However, the routine use of a biopsy for myocarditis is not standard, and similar results were found when we included only the biopsy-proven cases. In addition, we did not monitor all the changes in laboratory parameters between the start of ICI to myocarditis presentation because of the magnitude of effort. Some patients had one cycle of an ICI, some had several cycles, and we did not analyze the effect of ICI therapy on all the hematological parameters at each time point. The difference observed between cases and controls at baseline ALC raises the possibility that the changes observed over time in each cohort were a regression toward the mean. Other diseases could be confounding factors in the decrease of ALC and increase in NLR.[15,16] Additional research based on larger cohorts is needed to verify whether ALC and NLR measurements in ICI-associated myocarditis could be effectively used as part of the diagnostic algorithm. Approximately 47% of ICI-associated myocarditis cases also had other irAEs; however, similar results were found when we included only cases without other irAEs. This study does not provide insight into why the ALC or the NLR changes with myocarditis, and additional research is needed to understand the pathophysiological features behind lymphopenia in patients receiving ICI therapy, and the causality between lymphopenia and irAEs. However, we believe that these results are hypothesis generating, and further studies are needed. Recent data suggest that there may be an increase in atherosclerosis with ICIs.[32] Therefore, future studies will test the association between atherosclerotic cardiovascular events and the ALC and NLR. Finally, the modest number of events (n=20) precluded combining predictors and testing in a multivariable model the independent factors associated with MACEs with ICI-associated myocarditis.

CONCLUSIONS

A decline in ALC or increase in NLR during treatment with ICIs may be a marker for ICI-associated myocarditis. The greater change in both ALC and NLR correlated with a higher heart rate and lower blood pressure and, in follow-up, a >35% decrease in ALC or >100% increase in NLR was predictive of subsequent MACEs. The decline in ALC and the increase in NLR that occurs in patients with ICI-associated myocarditis could aid diagnosis and risk stratification in patients with ICI-associated myocarditis.

ARTICLE INFORMATION

Received July 7, 2020; accepted October 7, 2020.

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Acknowledgements

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Sources of Funding

This work was supported by National Institutes of Health/National Heart, Lung, and Blood Institute (T32HL076136, R01HL137562, R01HL130539, and K24HL150238 to Dr Neilan). Dr Neilan is also supported, in part, through a kind gift from A. Curtis Greer and Pamela Kohlberg.

Disclosures

Dr Neilan reports acting as a consultant for Parexel, H3 Biomedicine, Bristol Myers-Squibb, Abbvie, Astra Zeneca, and Intrinsic Imaging, unrelated to the current research. Dr Sullivan reports consulting/advisory board membership for Merck, Novartis, Bristol-Myers Squibb, and Replimune, and research support from Merck and Amgen. Dr Zhang reports consulting for Merck. The remaining authors have no disclosures to report.

Supplementary Material

Figures S1–S2

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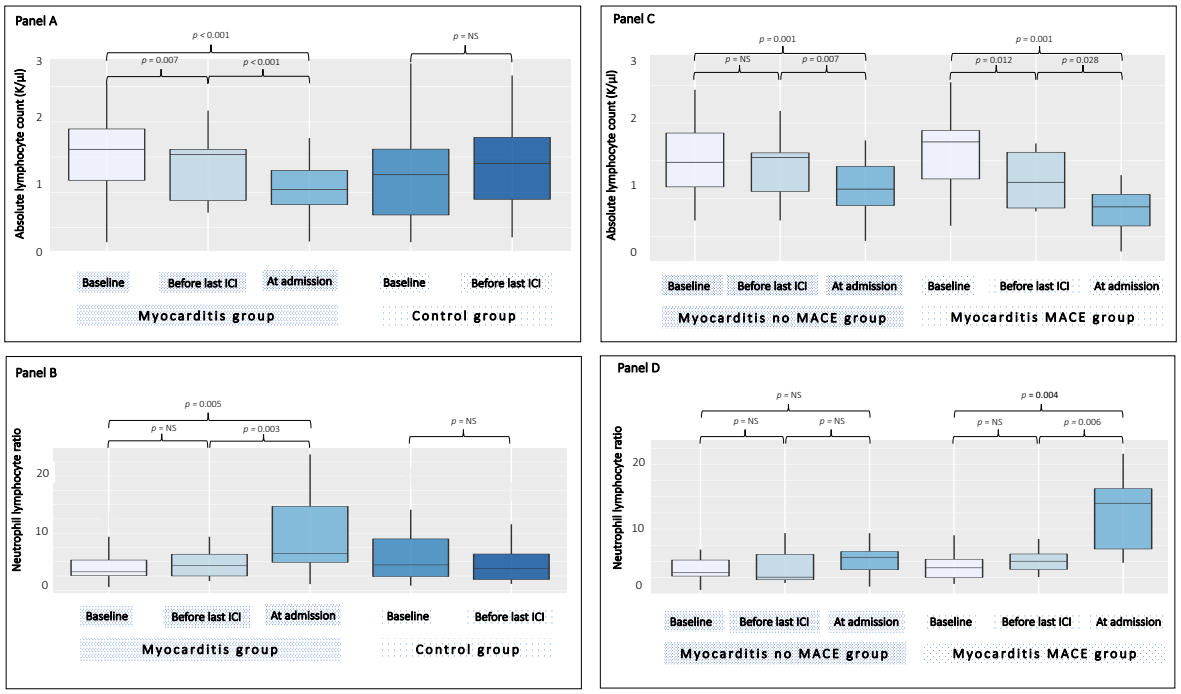
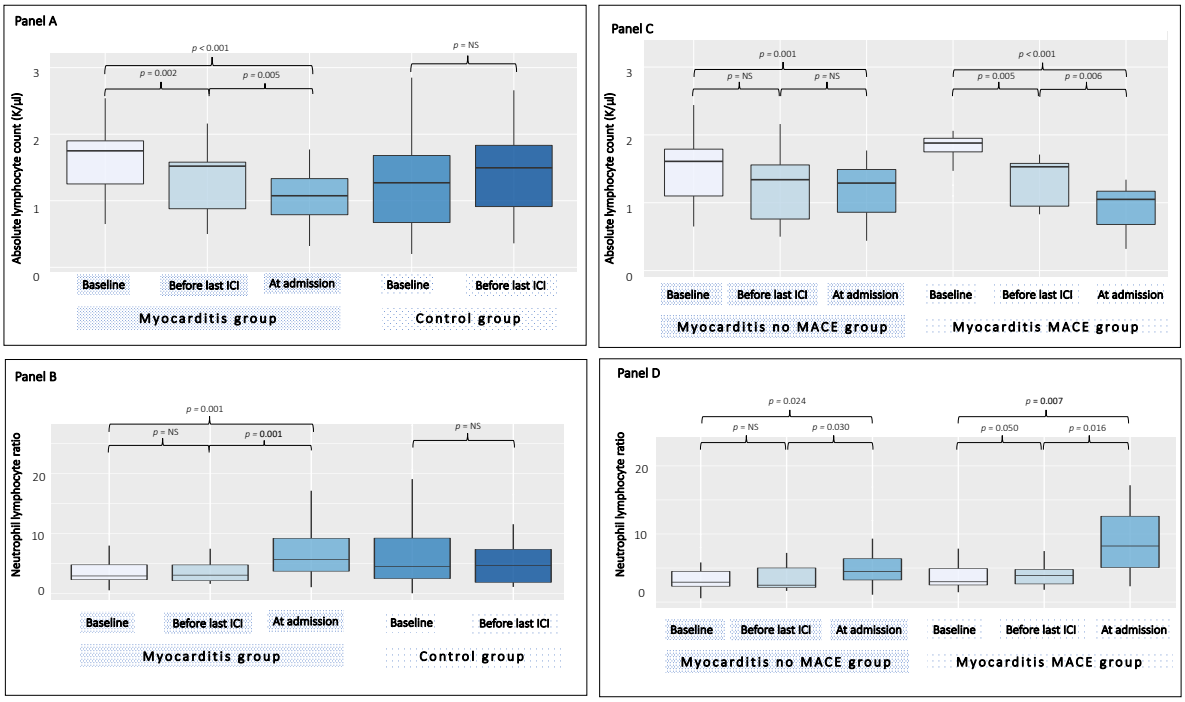
SUPPLEMENTAL MATERIAL

Figure S1: Barplots of absolute lymphocyte count (ALC) and neutrophil lymphocyte ratio (NLR) in immune checkpoint inhibitors (ICI)-associated myocarditis cases restricted to only biopsy proven cases (n = 31) and randomly selected control patients (n = 31) at several time points.



Panel A shows that ALC at baseline was higher among cases than controls (p = 0.03), followed by a decrease in the ALC from baseline, to prior last ICI dose, to presentation with myocarditis. In contrast, there was no change in the ALC over time in controls (Panel A). Panel B shows that among cases, the NLR at baseline was not different compared to controls (p = 0.46), followed by an increase in the NLR from baseline, to prior last ICI dose, to presentation with myocarditis. In contrast, the NLR did not change over time in controls (Panel B). Over a median follow-up period of 153 days, IQR 70–328 days, major adverse cardiac event (MACE) occurred in 13 cases (41.9%). Among those with a subsequent MACE, there was a greater percentage decrease in ALC (44.6% vs. 20.1%, p = 0.028) (Panel C) and a greater percentage increase in NLR (191.7 % vs 41.5%, p = 0.003) (Panel D).

Figure S2: Barplots of absolute lymphocyte count (ALC) and neutrophil lymphocyte ratio (NLR) in immune checkpoint inhibitors (ICI)-associated myocarditis cases restricted to patients with myocarditis and no other irAEs (n = 29) and controls (n = 55) at several time points.



Panel A shows that ALC at baseline was higher among cases the than controls (p = 0.006), followed by a decrease in the ALC from baseline, to prior last ICI dose, to presentation with myocarditis. In contrast, there was no change in the ALC over time in controls (Panel A). Panel B shows that among cases, the NLR at baseline was not different compared to controls (p = 0.45), followed by an increase in the NLR from baseline, to prior last ICI dose, to presentation with myocarditis. In contrast, the NLR did not change over time in controls (Panel B). Over a median follow-up period of 203 days, IQR 55–294 days, major adverse cardiac event (MACE) occurred in 13 cases (61.9%). Among those with a subsequent MACE, there was a greater percentage decrease in ALC (46.0% vs. 18.3%, p < 0.001) (Panel C) and a greater percentage increase in NLR (150.2 % vs 53.4%, p = 0.014) (Panel D).