Original Article

Prognostic Value of Troponin to Lymphocyte Ratio in Patients with Immune Checkpoint Inhibitor-associated Myocarditis

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Abstract

Background: **Immune checkpoint inhibitor (ICI)-associated myocarditis is a rare but life-threatening immune-related adverse event (irAE). Cardiac troponin (cTn) and absolute lymphocyte count (ALC) have been reported to be associated with ICI-myocarditis.**

Objective: **We aimed to investigate the prognostic value of the combination of these two features (cTn/ALC ratio) in ICI-associated myocarditis.**

Methods: **Our center performed a retrospective analysis of 46 patients with ICI myocarditis (cases) and 46 patients without myocarditis after receiving ICI (controls). We obtained data on cardiac enzymes, routine blood parameters, and other clinical biomarkers. Statistical significance was defined as a two-sided α level of 0.05. The association between these covariates and the clinical outcomes of ICI-mediated myocarditis was also tested.**

Results: **Abnormally elevated cTn ($p < 0.001$), cTn/ALC ratio ($p < 0.001$), and decreased ALC ($p < 0.001$) were observed in patients who developed ICI-myocarditis. The cTn/ALC ratio was associated with the development of severe myocarditis (OR, 5.05; 95% CI, 1.22-20.84; $p = 0.025$). Survival analysis and Cox regression analysis indicated that a higher cTn/ALC ratio emerged as a significant independent predictor for major adverse cardiac events (MACE) [HR, 5.64; 95%CI, 1.43-22.34; $p = 0.014$].**

Conclusions: **An increase in the cTn/ALC ratio was associated with the severity of ICI-myocarditis and could be an effective predictor of poor prognosis in patients with ICI-associated myocarditis.**

Keywords: **Immune Checkpoint Inhibitors; Myocarditis; Troponin; Lymphocyte Count; Prognosis.**

Introduction

Immune checkpoint inhibitor (ICI), which has been approved for first-line treatment in a multitude of cancers, has demonstrated remarkable efficacy.[1,2] However, ICI might be accompanied by a profile of autoimmune toxicities known as immune-related adverse events (irAEs).[3] A systematic review of randomized controlled trials showed that fatal irAEs occurred in 0.43% of patients receiving programmed cell death protein 1 (PD-1)/programmed cell death-ligand 1 (PD-L1) inhibitors, among which ICI-associated myocarditis (0.24%) was common.[4] Real-world consistent data have confirmed a higher incidence of myocarditis.[5,6] and a high fatality of ICI-mediated myocarditis, ranging from 35-50%.[7-9] Therefore, identifying prognostic biomarkers is crucial for promptly recognizing patients at risk of poor outcomes from ICI-associated myocarditis and providing early personalized therapy.

Detection of circulatory indicators could be a promising non-invasive method to investigate factors affecting the prognosis of patients with severe myocarditis after receiving ICI. Cardiac troponin (cTn), a biomarker of myocardial injury, is frequently linked to the severity and outcomes of ICI-associated myocarditis.[10-12] Recent data suggest that hematological inflammatory parameters such as absolute lymphocyte count (ALC) are associated with ICI-myocarditis and show an important prognostic role for subsequent major adverse cardiac events (MACE).[13,14] Since cTn is a cardiac-specific biomarker with high sensitivity and ALC is the only declined parameter that was significantly different between the myocarditis and non-myocarditis group, we wondered whether the integration of these two features—high cTn level and low ALC level—might

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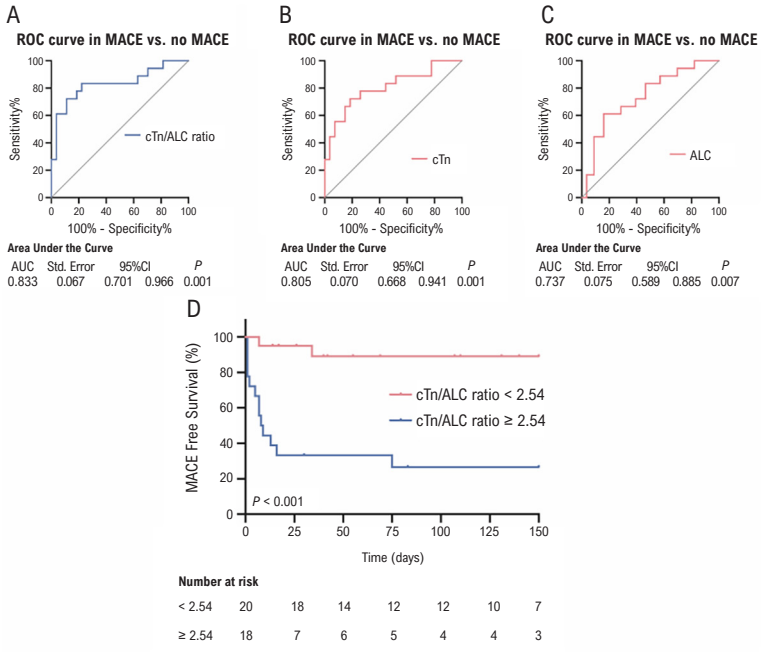
Central Illustration: Prognostic Value of Troponin to Lymphocyte Ratio in Patients with Immune Checkpoint Inhibitor-associated Myocarditis



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outperform either parameter alone. Therefore, we aimed to validate the hypothesis that the ratio of cTn to ALC (cTn/ALC ratio), expected to be high in the former and low in the latter, would improve the ability to predict the risk of poor outcomes in patients with ICI-mediated myocarditis by amplifying the significant difference. Few studies on the relationship between the cTn/ALC ratio and clinical outcomes of ICI-associated myocarditis have been reported. Therefore, we conducted a retrospective study of 46 patients with ICI-associated myocarditis and 46 controls to characterize the role of cTn/ALC ratio in ICI-myocarditis. The objective of this study was to evaluate the prognostic value of the cTn/ALC ratio in predicting clinical outcomes of patients with ICI-associated myocarditis.

Methods

Patient population

A retrospective, single-center, observational study was conducted at a large hospital. Patients who developed ICI-associated myocarditis (cases) from May 2019 to August 2022, including those who were transferred to our hospital because of myocarditis, were included in the study. Controls were randomly selected from all patients who started ICI therapy during the same time interval and were confirmed not to develop any irAEs at a ratio of 1:1. The random procedure was performed using a computer-generated random number sequence, ensuring that each eligible patient had an equal chance of being included in the control group. The exclusion criteria included lack of clinical data, other causes of myocarditis, patients with inflammatory comorbidities, patients with other relevant irAEs and infectious diseases, and patients with liver or kidney dysfunction. This study was reviewed and approved by the Medical Ethics Committee of a large hospital. The participants provided their written informed consent to participate in this study. The investigation conformed to the principles outlined in the Declaration of Helsinki.

Data Collection

Demographics (age, gender, and body mass index), cardiovascular risk factors, malignancy status (cancer type and clinical stage), cancer medications (prior tumor therapy and ICI treatment status), clinical outcomes (MACE and survival), and hematological parameters (cardiac biomarkers and blood cells) were retrospectively extracted from electronic medical records. Data were recorded at the time of ICI-associated myocarditis onset in the myocarditis group and before the last ICI dose in the controls.

Diagnosis and definitions

Diagnosis of ICI myocarditis was performed by experienced oncologists and cardiologists based on the clinical criteria of the European Society of Cardiology guidelines or standard

histological features present in endomyocardial biopsy.[15] ICI-related myocarditis grading was performed using the Common Toxicity Criteria for Adverse Events (version 5.0).[16] Cases were categorized as severe myocarditis if they displayed grade ≥ 3 myocarditis, while mild myocarditis was defined as grade ≤ 2 myocarditis. MACE was defined as a composite of cardiovascular death, cardiac arrest, cardiogenic shock, and hemodynamically significant complete heart block.[8] MACE-free survival of ICI-related myocarditis patients was calculated from the diagnosis of ICI-associated myocarditis until MACE or the last follow-up date. The cTn/ALC ratio was calculated using the following equation: cTn/ALC ratio = cTn (pg/mL)/ALC (K/μL).

Statistical analysis

Continuous variables were summarized as mean ± standard deviation or median with interquartile range (IQR). The normality of continuous variables was assessed using the Shapiro-Wilk test. Categorical variables were expressed as frequencies and percentages. Continuous variables were compared using either an independent-sample t-test or the Mann-Whitney U test. Chi-square ($\chi 2$) or Fisher's exact test was used to analyze categorical variables. Pearson correlation analysis was performed to assess the linear relationships between cTn/ALC and NT-proBNP, as well as between cTn/ALC and other clinical laboratory markers. The correlation coefficients and corresponding p-values were calculated to determine whether significant associations exist between the variables. Receiver-operating characteristic (ROC) curves were constructed to assess the discriminatory ability of the cTn/ALC ratio for diagnosis, differentiation of severity, and MACE prediction and to calculate the optimal cut-off value using the Youden Index. The statistical significance of differences between AUCs was evaluated using the DeLong test, implemented in SPSS (version 27.0). Univariable and multivariable logistic regression analyses were performed to identify factors associated with severe myocarditis, with odds ratios (OR) and 95% confidence intervals (CI) calculated using maximum likelihood estimation. Additionally, binary logistic regression analysis was conducted to assess the potential interactions between cTn/ALC, NT-proBNP, and other clinical laboratory markers in predicting major adverse cardiovascular events (MACE). Interaction terms were included in the regression model. Kaplan-Meier curves were used to quantify the relationship between the cTn/ALC ratio and MACE-free survival. Univariable and multivariable Cox proportional hazard models were constructed to determine the prognostic factors associated with MACE-free survival in patients with myocarditis, with hazard ratios (HR) and 95% confidence intervals (CI) estimated based on the partial likelihood function. All statistical tests were two-tailed with a $P < 0.05$ level of significance. Statistical analyses were conducted using SPSS Statistics 26.0 and GraphPad Prism 9.4. Figures were created using GraphPad Prism 9.4 and Microsoft PowerPoint 16.71.

Results

Study population and patient characteristics

A total of 46 patients clinically diagnosed with ICI-associated myocarditis were included in the study, and only one case was confirmed pathologically (Supplementary Table 1). Given the rarity of ICI-associated myocarditis and the limited availability of data, we included all eligible cases identified during the study period (convenience sampling). The baseline clinical characteristics of all patients are shown in Table 1. Among 46 patients with ICI-associated myocarditis, the mean age was 60 ± 11 years, and 76.1% were male. Patients with cardiac risk factors accounted for 50.0% of cases, among which smoking accounted for 32.6%, followed by hypertension (21.7%), which did not differ significantly between the myocarditis and control groups.

The most common indication for ICI in our cohort was lung cancer. Most patients in both groups were treated with PD-1 inhibitors. In addition, combination therapy (ICI combined with chemotherapy or ICI combined with targeted therapy) was the predominant treatment type in both groups.

Comparison of laboratory examination results in the myocarditis group and control group

We compared the clinical examination results of patients with myocarditis with those of the controls. The cTn level on presentation was significantly higher in patients with ICI-myocarditis than in controls, while a significant decrease was observed in ALC in the myocarditis group. An increase in the cTn/ALC ratio was observed in the myocarditis group (Table 2).

Comparison of laboratory examination results in severe myocarditis group and mild myocarditis group

In the myocarditis group, 31 patients (67.3%) experienced severe ICI-associated myocarditis. At the time of presentation, the median cTn was 2.19 pg/mL in the severe myocarditis group and 0.03 pg/mL in the mild myocarditis group. On the contrary, patients with severe myocarditis had lower ALC on presentation compared with patients with mild myocarditis. The cTn/ALC ratio significantly increased in the severe myocarditis group. Univariate and multivariate logistic regression analysis demonstrated that a higher cTn/ALC ratio was independently and positively correlated with severe myocarditis (Table 3 and Table 5).

Correlation of troponin to lymphocyte ratio and prognosis

During a median follow-up of 69 days (IQR: 17-227), MACE occurred in 18 cases (39.1%) and 16 out of 46 (34.8%) (Supplementary Table 2). ROC curves were used to calculate the optimal cut-off point for predicting MACE in patients with myocarditis. To assess the predictive value of the cTn/ALC ratio for outcomes in patients with ICI-mediated myocarditis, ROC curves for cTn/ALC ratio, cTn, and ALC were compared. In multiple ROC curves for identifying patients who developed MACE, the cTn/ALC ratio showed better performance than cTn or ALC (Central Illustration). However, the DeLong test revealed no statistically significant differences in AUC between the cTn/ALC ratio and cTn ($p = 0.77$) or ALC ($p = 0.30$). Based

Table 1 – Clinical characteristics of myocarditis cases and controls

	Cases (n=46)	Controls (n=46)	p-value
Age, y, mean ± SD	60±11	61±8	0.731
Male sex, n (%)	35 (76.1)	35 (76.1)	1.000
BMI, kg/m², mean ± SD	22.2±3.5	23.4±3.2	0.109
Cardiovascular risk factors			
Smoking (current or past), n (%)	15 (32.6)	19 (41.3)	0.388
Diabetes mellitus, n (%)	5 (10.9)	7 (15.2)	0.536
Hypertension, n (%)	10 (21.7)	8 (17.4)	0.599
Hyperlipidemia, n (%)	1 (2.2)	1 (2.2)	1.000
No cardiovascular risk factors, n (%)	23 (50.0)	22 (47.8)	0.835
Primary cancer type			
Lung cancer, n (%)	36 (78.3)	45 (97.8)	0.004
Gastrointestinal tumors, n (%)	2 (4.3)	1 (2.2)	1.000
Thymoma, n (%)	5 (10.9)	0 (0.0)	0.066
Hepatoma, n (%)	2 (4.3)	0 (0.0)	0.475
Renal cancer, n (%)	1 (2.2)	0 (0.0)	1.000
Prior cancer treatment			
Chemotherapy, n (%)	19 (41.3)	18 (39.1)	0.832
VEGF or EGFR inhibitors, n (%)	9 (19.6)	13 (28.3)	0.328
Radiation, n (%)	5(10.9)	2 (4.3)	0.432
Surgery, n (%)	16 (34.8)	4 (8.7)	0.002
ICI treatment			
ICI monotherapy, n (%)	11 (23.9)	1 (2.2)	0.002
ICI combines chemotherapy, n (%)	31 (67.4)	43 (93.8)	0.002
ICI combines VEGF or EGFR inhibitors, n (%)	7 (15.2)	12 (26.1)	0.198
ICI type			
Any PD-1	44 (95.7)	46 (100.0)	0.475
Any PD-L1	2 (4.3)	0 (0.0)	0.475

BMI: body mass index; VEGF: Vascular Endothelial Growth Factor; EGFR: Epidermal Growth Factor Receptor; ICI: Immune checkpoint inhibitor.

on ROC curve analysis, it was determined that the optimal cut-off value of the cTn/ALC ratio was 2.54 by means of the Youden Index. Myocarditis patients were classified into two groups [low cTn/ALC ratio group (cTn/ALC ratio<2.54) and high cTn/ALC ratio group (cTn/ALC ratio ≥ 2.54)] according to the cut-off value and the Kaplan-Meier curve was generated. A significantly lower MACE-free survival curve was observed in patients with a cTn/ALC ratio ≥ 2.54 (Central Illustration). No significant linear relationship or potential interaction was found between the cTn/ALC ratio and NT-proBNP or other variables (Supplementary Tables 3 and 4).

To determine whether the cTn/ALC ratio was an independent predictor of MACE in patients who suffered from ICI-associated myocarditis, we also performed univariate and multivariate Cox regression analysis (Table 4 and Table 6). Patients with myocarditis were divided into a high group and a low group according to the optimal cut-off value of each covariate obtained using ROC curves. Univariate Cox regression analysis showed that patients with a higher cTn/ALC ratio (≥ 2.54) had a worse prognosis. In multivariate Cox regression analysis, a higher cTn/ALC ratio was significantly and independently associated with a trend toward reduced MACE-free survival in patients with ICI-related myocarditis. Therefore, the cTn/ALC ratio remained a strong independent predictor of MACE-free survival in patients with ICI-mediated myocarditis after adjusting for other confounding factors. These data suggested the value of the cTn/ALC ratio for predicting MACE in patients with ICI-mediated myocarditis.

Table 2 – Laboratory examination results on presentation in patients with immune checkpoint inhibitor associated myocarditis and controls

	Cases (n=46)	Controls (n=46)	p-value
cTn (pg/mL)	0.88 (0.20-4.57)	0.00 (0.00-0.01)	<0.001
NT-proBNP (pg/mL)	1326.25 (335.90-6198.00)	83.37 (28.42-240.00)	<0.001
CK (U/L)	659.75 (205.70-3451.40)	62.50 (41.45-89.15)	<0.001
CK-MB (U/L)	62.50 (27.00-123.00)	10.00 (7.00-14.00)	<0.001
ALC (K/μL)	0.50 (0.30-0.90)	1.55 (1.00-2.00)	<0.001
AMC (K/μL)	0.50 (0.30-0.80)	0.65 (0.50-0.90)	0.041
ANC (K/μL)	11.85 (7.20-16.20)	4.25 (3.00-5.60)	<0.001
PLT (K/μL)	179.00 (96.00-254.00)	229.00 (187.00-318.00)	0.017
cTn/ALC ratio	2.47 (0.30-11.73)	0.00 (0.00-0.01)	<0.001

Table 3 – Laboratory examination results on presentation in patients with mild myocarditis and severe myocarditis

	Severe myocarditis (n=31)	Mild myocarditis (n=15)	p-value
cTn (pg/mL)	2.19 (0.48-9.92)	0.03 (0.01-0.26)	<0.001
NT-proBNP (pg/mL)	3000.50 (528.50-9049.00)	282.05 (22.20-860.90)	0.004
CK (U/L)	991.35 (300.20-4013.10)	280.80 (154.05-2147.75)	0.089
CK-MB (U/L)	87.50 (44.00-141.00)	23.00 (16.00-38.00)	0.001
ALC (K/μL)	0.35 (0.20-0.70)	0.90 (0.60-1.30)	0.002
AMC (K/μL)	0.55 (0.30-1.00)	0.40 (0.25-0.65)	0.228
ANC (K/μL)	13.65 (9.20-17.40)	4.45 (3.25-11.40)	0.002
PLT (K/μL)	168.50 (79.00-255.00)	207.00 (168.50-227.00)	0.423
CRP(mg/L)	45.8 (13.84-116.90)	43.26 (14.23-116.29)	0.235
IFN-γ (pg/mL)	1.58 (1.01-3.59)	1.38 (0.34-2.82)	0.974
TNF-α (pg/mL)	1.23 (0.77-3.54)	1.22(0.65-3.59)	0.335
Duration of hormone therapy (day)	23 (4-37)	11 (3-24)	0.115
Total dose of hormone therapy (mg)	1500.00 (570.00-3584.00)	1000.00(600.00-3180.00)	0.108
cTn/ALC ratio	4.87 (1.98-19.84)	0.07 (0.00-0.58)	<0.001

Discussion

In this retrospective study, we investigated the prognostic value of the cTn/ALC ratio in predicting outcomes in patients with myocarditis following ICI therapy. We found that, among patients who developed myocarditis after ICI treatment, higher cTn/ALC ratios at the time of onset were associated with more severe clinical manifestations and worse prognosis. Specifically, the cTn/ALC ratio can effectively and independently predict the severity and prognosis of ICI-related myocarditis. The cTn/ALC ratio integrates two distinct but complementary biomarkers: cardiac troponin (cTn), a specific marker of myocardial injury, and ALC, which reflects the systemic immune status and is often reduced in severe cases. Compared to traditional biomarkers cTn or ALC, cTn/ALC not only accounts for immune dysregulation (ALC) but also amplifies the signal of myocardial injury (cTn). As a composite biomarker, the cTn/ALC ratio provides an earlier and more comprehensive reflection of the patient's pathophysiological state. Our study establishes the cut-off value of the cTn/ALC ratio for predicting the prognosis of immune-related myocarditis. When the cTn/ALC ratio exceeds this threshold, the risk of poor prognosis significantly increases. Therefore, this threshold can be used for risk stratification in patients with immune-related myocarditis, helping clinicians to identify high-risk MACE patients promptly. Given the wide availability, standardization, low cost, minimally invasive nature, and ease of interpretation of both cTn and ALC measurements, the cTn/ALC ratio may serve as a clinically feasible predictive marker for the severity and prognosis of ICI-induced myocarditis.

Table 4 – Laboratory examination results on presentation in patients with prognosis in immune checkpoint inhibitor associated myocarditis

	MACE (n=18)	MACE free (n=28)	p-value
cTn (pg/mL)	4.57 (0.41-22.27)	0.26 (0.04-2.28)	0.017
NT-proBNP (pg/mL)	4848.00 (3775.00-9120.00)	1746.00 (334.80-5604.00)	0.031
CK (U/L)	2218.80 (457.85-3305.20)	407.2 (183.10-1965.50)	0.121
CK-MB (U/L)	109.00 (82.00-207.50)	47.00 (17.00-120.00)	0.009
ALC (K/μL)	0.60 (0.40-1.05)	1.00 (0.70-1.20)	0.002
AMC (K/μL)	0.30 (0.10-0.65)	0.30 (0.30-1.20)	0.409
ANC (K/μL)	7.60 (4.45-11.30)	8.60 (3.80-10.30)	0.676
PLT (K/μL)	247.00 (129.50-337.50)	105.00 (213.00-299.00)	0.397
IFN-γ (pg/mL)	2.98 (0.49-3.68)	1.55 (1.15-3.15)	0.620
TNF-α (pg/mL)	0.86 (0.53-3.88)	1.23 (0.88-2.88)	0.538
CRP (mg/L)	46.80 (23.03-122.92)	38.04 (9.59-116.29)	0.142
Duration of hormone therapy (day)	17.00 (4.00-39.00)	21.00 (5.00-32.00)	0.554
Total dose of hormone therapy (mg)	1686.00 (932.00-3130.00)	1000.00 (570.00-3910.00)	0.181
cTn/ALC ratio	6.53 (0.68-34.18)	0.37 (0.04-2.38)	0.010

MACE: major adverse cardiac events.

Table 5 – Logistic regression analysis of Laboratory examination results associated with severity of immune checkpoint inhibitor associated myocarditis

	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	p-value	OR (95% CI)	p-value
NT-proBNP (pg/mL)	1.00 (1.00-1.00)	0.074	-	-
CK (U/L)	1.00 (1.00-1.00)	0.232		
CK-MB (U/L)	1.01 (0.99-1.02)	0.157	-	-
AMC (K/μL)	1.47 (0.34-6.25)	0.606	-	-
ANC (K/μL)	1.27 (1.08-1.49)	0.005	1.23 (1.00-1.50)	0.045
PLT (K/μL)	0.99(0.98-1.00)	0.609	-	-
CRP(mg/L)	1.00 (0.98-1.02)	0.412	-	-
IFN-γ (pg/mL)	1.79 (0.50-6.38)	0.368	-	-
TNF-α (pg/mL)	0.64 (0.22-1.84)	0.416	-	-
Duration of hormone therapy (day)	1.06 (0.97-1.16)	0.181	-	-
Total dose of hormone therapy (mg)	1.00 (0.99-1.01)	0.695	-	-
cTnI/ALC ratio	4.66 (1.23-17.59)	0.023	5.05 (1.22-20.84)	0.025

In patients diagnosed with ICI myocarditis, more than half of cases would progress to severe myocarditis, which is associated with poor prognosis of ICI-mediated myocarditis.[11,17] Consistent data have established that early identification of severe myocarditis followed by high-dose and early corticosteroid intervention could significantly reduce the occurrence of MACE.[17,18] Therefore, indicators to identify the severity of ICI-myocarditis are urgently needed. Both Lei and Puzanov10, 11 have reported differences between mild and severe myocarditis. Both studies found that troponin levels were significantly elevated in patients with severe ICI-myocarditis. However, they did not find significant differences in blood cells, including lymphocytes.[10,11] In contrast, our study found that ALC was significantly associated with the severity of ICI-myocarditis. In our analysis, we found not only differences in cardiac troponin (cTn) and lymphocyte levels between the

Table 6 – Cox proportional regression analysis of laboratory examination results associated with prognosis in immune checkpoint inhibitor associated myocarditis

	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	p-value	HR (95% CI)	p-value
NT-proBNP (<2872.50 vs. ≥2872.50, pg/mL)	4.46 (1.43-13.93)	0.010	6.25 (1.63-23.94)	0.008
CK (<426.85 vs. ≥426.85, U/L)	5.68 (1.29-25.07)	0.022	-	-
CK-MB (<62.50 vs. ≥62.50, U/L)	6.09 (1.70-21.73)	0.005	-	-
ANC (<8.90 vs. ≥8.90, K/μL)	13.02 (1.6-100.94)	0.014	16.37 (1.75-153.01)	0.014
AMC (<0.55 vs. ≥0.55, K/μL)	1.09(0.45-2.63)	0.843	0.08(0.01-1.16)	0.064
PLT (<222.50 vs. ≥222.50, K/μL)	1.00(0.99-1.00)	0.568	0.99(0.98-1.02)	0.905
CRP (<29.42 vs. ≥29.42, mg/L)	1.00(0.98-1.01)	0.797	1.02(0.98-1.04)	0.370
IFN-γ (<2.85 vs. ≥2.85, pg/mL)	1.08(0.72-1.34)	0.921	0.901(0.47-1.77)	0.779
TNF-α(<2.67 vs. ≥2.67, pg/mL)	1.20(0.74-1.93)	0.444	1.08(0.45-2.62)	0.863
Duration of hormone therapy (<28 vs. ≥28, day)	0.99(0.94-1.04)	0.775	1.00(0.91-1.09)	0.966-
Total dose of hormone therapy (<1276 vs. ≥1276 mg)	1.00(0.99-1.00)	0.999	1.00(1.00-1.00)	0.394
cTn/ALC ratio (<2.54 vs. ≥2.54)	7.62 (2.15-26.96)	0.002	5.64 (1.43-22.34)	0.014

mild and severe myocarditis groups but also a significant increase in the cTn/ALC ratio, which combines high cTn and low ALC in the severe myocarditis group. Univariate and multivariate analyses showed that the cTn/ALC ratio was independently associated with severe ICI-related myocarditis.

Meanwhile, some studies have suggested that NT-proBNP is a marker of prognosis and severity in heart failure, and it could potentially serve as a marker for the severity and prognosis of myocarditis as well.[19,20] Therefore, we also collected and analyzed NT-proBNP data from these myocarditis patients. However, our analysis found that NT-proBNP is not strongly associated with the severity of immune-related myocarditis (Table 5). It is suggested that NT-proBNP cannot predict the severity of immune-related myocarditis, whereas the cTn/ALC ratio identified in this study can predict the severity of immune-related myocarditis.

Early identification and targeted treatment of patients at high risk of MACE are also crucial for the improvement of prognosis. An inexpensive and widely available biomarker that could stratify the risk of poor prognosis at the time of diagnosis may be useful. Troponin is a highly specific indicator of cardiac tissue damage and can be detected in serum. In a study by Waissengein, MACE was found to be more common in patients with an elevated baseline cTn in patients with cardiovascular irAEs, including myocarditis. In addition, elevated follow-up cTn level emerged as a significant independent predictor for the development of adverse outcomes.[21] Similarly, a study by Puzanov showed that a higher cTn level was associated with increased mortality.[11] Peripheral blood cell examination is a routine procedure for patients with cancer before and after ICI administration; therefore, peripheral blood cells are frequently used as prognostic indicators for patients with cancer. A retrospective study by Drobni et al. demonstrated that there was a statistically significant decrease in ALC from baseline to before the last ICI dose to presentation with myocarditis in patients with ICI-associated myocarditis. Moreover, a greater decrease in ALC was associated with the occurrence of subsequent MACE. An ALC reduction > 35% was associated with worse outcomes after ICI-mediated myocarditis, suggesting that ALC measurement could be used as a diagnostic algorithm in ICI-associated myocarditis.[13] Another study conducted by Xie et al. showed that the ALC level at presentation was lower than the baseline ALC level in patients with ICI-associated myocarditis[17] (Table 7). In our study, we observed elevated cTn levels and reduced ALC levels in the MACE group. More importantly, the ratio of these two markers (cTn/ALC ratio) showed a significant correlation with the prognosis of ICI-related myocarditis. Based on ROC analysis and survival analysis, the cTn/ALC ratio demonstrated a higher predictive value for MACE compared to either cTn or ALC alone. The optimal cut-off value for the cTn/ALC ratio to assess MACE risk was defined as 2.54. It is suggested that the cTn/ALC ratio, as a predictor of the prognosis of immune-related myocarditis, has better clinical value compared to cTn and ALC as individual markers. Nevertheless, in addition to cTn and ALC, some studies have suggested that NT-proBNP is not only a prognostic marker for heart failure but may also be a relevant indicator for the prognosis of myocarditis.[19,20] Therefore, we also collected NT-proBNP data from patients with immune-related myocarditis and analyzed its correlation with prognosis. We found that NT-proBNP is also associated with the prognosis of immune-related myocarditis patients (Table 6), and thus, we performed ROC curve analysis. The results showed that, compared to NT-proBNP, the cTn/ALC ratio has superior predictive value

Table 7 – Summary of studies on the role of absolute lymphocyte count (ALC) and/or cTnI in immune checkpoint inhibitor (ICI) associated myocarditis

N	Study design	Number of patients with ICI-associated myocarditis	Main finding of ALC	Main finding cTn	Publish year
1[22]	Retrospective study	12	Lymphopenia was found in 66.7% of patients with cardiac irAEs (66.7%)	All patients had elevations in cTn level.	2023
2[21]	Retrospective study	81	NA	Troponin was associated with severe myocarditis, and MACE—peak troponin levels after myocarditis were related to myocarditis severity.	2023
3[23]	Prospective study	60	NA	Within 72 hours of admission, cTn was increased in patients with myocarditis compared with the upper reference limit with varying degrees.	2023
4[11]	Retrospective study	15	NA	Higher cTn level on presentation correlated with progression to severe myocarditis. Higher cTn level was associated with increased mortality.	2021
5[13]	Retrospective study	55	In some cases, there was a statistically significant decrease in ALC from baseline to before the last ICI dose to presentation with myocarditis. A greater decrease in ALC was also associated with the occurrence of subsequent MACE.	NA	2020
6[17]	Retrospective study	46	ALC level on presentation in patients with ICI-associated myocarditis was lower than the baseline ALC level.	NA	2022

for MACE events. These findings indicate that compared to cTn, ALC, and NT-proBNP, the cTn/ALC ratio is a more effective and clinically valuable predictor of prognosis in patients with immune-related myocarditis.

This study has several limitations that should be acknowledged. First, the retrospective and single-center design introduces potential selection bias. Our convenience sampling approach (including all eligible cases during the study period) may not fully represent the broader population of ICI-associated myocarditis, especially given the rarity of this condition. Additionally, the control group, although randomly selected, was derived from the same institution, which might limit the generalizability of our findings. Second, the small sample size (n=46) and short follow-up duration (median 69 days) restrict the statistical power to detect subtle associations and long-term outcomes. They may also contribute to the wider confidence intervals observed in our logistic regression and Cox regression analyses. While our findings suggest a strong association between the cTn/ALC ratio and outcomes, the wide CI emphasizes the need for further validation in larger cohorts. Therefore, we will conduct future multicenter studies with larger cohorts and longer follow-ups to validate our findings and refine the prognostic utility of the cTn/ALC ratio.

Conclusions

In our cohort, the increase in the cTn/ALC ratio that occurred in patients with ICI-associated myocarditis could contribute to risk stratification in patients with ICI-associated myocarditis. The cTn/ALC ratio at presentation has emerged as a significantly strong independent predictor of the prognosis in patients with ICI-associated myocarditis.

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Author Contributions

Conception and design of the research: Chen W, Lin X, Zhou C, He Y; Acquisition of data: Chen W, Zhang W, Luo C, Cai J; Analysis and interpretation of the data: Chen W, Zhang W, Luo C; Statistical analysis: Chen W, Zhang W, Luo C; Writing of the manuscript: Chen W; Critical revision of the manuscript for content: Zhang W, Zhou C, He Y.

Potential conflict of interest

No potential conflict of interest relevant to this article was reported.

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Study association

This study is not associated with any thesis or dissertation work.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the The Medical Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University under the protocol number 2022-80. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

Use of Artificial Intelligence

The authors did not use any artificial intelligence tools in the development of this work.

Data Availability

Due to the presence of sensitive clinical information and ongoing data curation, the full dataset is not publicly available at this stage.However, data necessary to evaluate the findings of this study can be made available to reviewers upon request, under confidentiality and ethical compliance.

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*Supplemental Materials

For additional information Supplemental Material 1, please click here.

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