The Intrinsic Differences Between Immune Checkpoint Inhibitor Induced Myocarditis: A Retrospective Analysis of Real-World Data

Yanna Lei

West China Hospital of Sichuan University

Xiufeng Zheng

West China Hospital of Sichuan University

Qian Huang

West China Hospital of Sichuan University

Xiaoying Li

West China Hospital of Sichuan University

Meng Qiu

West China Hospital of Sichuan University

Ming Liu (✉ liuming629@wchscu.cn)

West China Hospital of Sichuan University

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Abstract

Immune-related myocarditis is a severe and even life-threatening immune-related adverse event (irAE) which may also be under-estimated due to the challenge in diagnosis. There have been few reports about the intrinsic difference between patients with immune-associated myocarditis. A retrospective analysis was conducted between March 2019 to June 2020 in West China Hospital and 18 patients with immune-related myocarditis were studied. Cases were classified as mild (n=12) or severe myocarditis (n=6) according to the clinical manifestations and haemodynamic complications. Covariates extracted from medical records were compared in different groups, and factors associated with severe myocarditis were identified. In this retrospective analysis, the median age of the 18 patients was 60 years old. Most cases occur early and approximately after the first or second ICI infusion. The severity of myocarditis may be correlated with lactate dehydrogenase (LDH) ($P=0.04$) and troponin ($P=0.0057$). The relationship between troponin and myocarditis was further confirmed in another cohort which including 30 patients. In addition, patients are more likely to develop multi-irAEs, and myositis was the most common second irAE. Those who experience multi-irAEs had significantly higher LDH ($P=0.02$) as well as myoglobin levels ($P=0.02$) than did not experience. All patients were treated with steroids timely and the mortality rate was 5.6% in our study. In this study, we explore risk factors for severe myocarditis and we emphasized the importance of a multidisciplinary team in assisting diagnosis and treatment options. It is critical to initiate corticosteroid therapy, regardless of the severity of the myocarditis.

1. Introduction

The advent of immune-checkpoint inhibitors (ICIs) which blocking cytotoxic T lymphocyte associated protein-4 (CTLA-4) and the programmed death-1 (PD-1)/programmed death ligand-1 (PD-L1) axis, both as monotherapy or in combination strategies, have revolutionized cancer treatment¹⁻³. However, as the use of ICIs in clinical practice has increased, immune-mediated toxicities have been observed, which can impact numerous organ systems, including the heart^{4,5}. Immune-related myocarditis is the most serious life-threatening toxicity and the underlying pathomechanism is poorly understood⁶. The incidence of immune-related myocarditis caused by ICI is approximately 1%, but the mortality rate can be as high as 46%^{7,8}. Because of its rarity, information on this unusual disease is limited, and further research is needed to fully understand the devastating irAEs. Furthermore, there is a scarcity of patients' data on the clinical characteristics, results of pertinent examinations, and outcomes of ICI-related cardiotoxicity.

To gain a better knowledge of this disease, we analyzed and described the characteristics of patients with immune-related myocarditis, including baseline clinical characteristics, drug-related data, relevant examination results, and outcomes. Our research will look into the internal disparities between patients.

2. Materials And Methods

2.1 Study population

In this single-center retrospective study, data were collected from 18 patients with ICI-related myocarditis in West China Hospital from March 2019 to June 2020. Inclusion criteria were listed as follows: (1) a histologically confirmed diagnosis of solid malignancy in West China Hospital;(2) age ≥ 18 years at ICI initiation; (3) received at least one cycle of ICI;(4) met the diagnostic criteria through multidisciplinary panel decision;(5) exclude the possible diagnosis of coronary artery disease and myocarditis caused by other reasons. The study was approved by the institutional review board, and the requirement for written informed consent was waived. The present study was approved by the institutional review board of West China Hospital and was exempted from informed consent requirements owing to its retrospective design.

This article does not contain any studies with human participants or animals performed by any of the authors. All methods were performed in accordance with the relevant guidelines and regulations.

2.2 Patients data collection

The following characteristics of patients were recorded: (1) clinical baseline characteristics: age, gender, smoking and alcohol status, comorbid conditions, disease stage, cancer type and histological subtype et.al; (2) ICI-related information: type of ICI, treatment start date, the date of diagnosis, therapy line, combination with other drugs, response patterns; (3) relevant examination results: laboratory data including myocardial markers and infection-related indicators, echocardiography, coronary angiography, coronary computed tomography (CT) et.al; (4) treatment and outcome.

2.3 Statistical Analysis

Study variables were presented as number (n) – percentage (%) and mean $\pm$ standard deviation. Wilcoxon rank-sum tests are used for continuous variables and χ^2 or Fisher's exact test is used for categorical variables. Missing data will not be analyzed in this study. The statistical analyses were conducted via R 4.0.3 and GraphPad Prism 8. $P < 0.5$ was a statistically significant standard.

3. Results

3.1 Baseline Characteristics

Among the 18 patients who met the selection criteria were retrospectively identified in this study, and the patients were classified into mild (n=12) and severe myocarditis (n=6) according to the clinical manifestation and whether they had haemodynamic complications such as heart failure, cardiogenic shock, or arrhythmia. The clinical characteristics of the 18 patients with immune-related myocarditis are shown in Table 1.

Table 1

Clinical characteristics of the patients and information of ICI in patients with immune-related myocarditis.

	Mild group (N=12)	Severe group (N=6)	All Cases (N=18)	P
Age(years)				0.08
Median [Min, Max]	61.5 [45.0, 79.0]	44.5 [29.0, 67.0]	60.0 [29.0, 79.0]	
Gender				1.00
Male	10 (83.3%)	5 (83.3%)	15 (83.3%)	
Female	2 (16.7%)	1 (16.7%)	3 (16.7%)	
Smoking history				0.60
Yes	5 (41.7%)	1 (16.7%)	6 (33.3%)	
No	7 (58.3%)	5 (83.3%)	12(66.7%)	
Alcohol history				1.00
Yes	2 (16.7%)	1 (16.7%)	3 (16.7%)	
No	10(83.3%)	5 (83.3%)	15(83.3%)	
Diabetes				1.00
Yes	3 (25.0%)	2 (33.3%)	5 (27.8%)	
No	9 (75.0%)	4 (66.7%)	13 (72.2%)	
Hypertension				0.04
Yes	7 (58.3%)	0 (0%)	7 (38.9%)	
No	5 (41.7%)	6 (100%)	11 (61.1%)	
Cancer type				0.048
lung cancer	9 (75.0%)	1 (16.7%)	10 (55.6%)	
thymic carcinoma	1 (8.3%)	3 (50.0%)	4 (22.2%)	
Other cancers	2(16.7%)	2 (33.3%)	4 (22.2%)	
Cancer stage				0.24
Stage IV	8(66.7%)	5(83.3%)	13(72.2%)	

ICI, immune checkpoint inhibitor; PD, progressive disease; PD-L1, programmed death-ligand 1; PR, partial response, SD, stable disease; PD, progressive disease. Tumour responses were assessed using computer tomography and determined using RECIST version 1.1.

	Mild group (N=12)	Severe group (N=6)	All Cases (N=18)	P
Stage III	4 (33.3%)	1 (16.7%)	5 (27.8%)	
Clinical symptoms				0.141
Yes	3(25.0%)	6(100%)	9(50%)	
No	9 (75.0%)	0(0%)	9(50%)	
Initial treatment line				0.01
1st	9 (75.0%)	2 (33.3%)	11 (61.1%)	
2nd	2 (16.7%)	0 (0%)	2 (11.1%)	
≥3rd	0 (0%)	4 (66.7)	4 (22.2%)	
Missing	1 (8.3%)	0 (0%)	1 (5.6%)	
ICI type				1.00
Anti-PD-1	10 (83.3%)	5 (83.3%)	15 (83.3%)	
Anti-PD-L1	2 (16.7%)	1 (16.7%)	3 (16.7%)	
Treatment category				0.42
ICI alone	6 (50%)	3 (50.0%)	9(50.0%)	
Chemo + ICI	6 (50%)	2 (33.3%)	8(44.4%)	
Targeted + ICI	0 (0%)	1 (16.7%)	1 (5.6%)	
Times to onset				0.40
Mean (SD)	64.8 (52.3)	36.2 (20.6)	55.2 (45.7)	
Median [Min, Max]	57.0 [4.00, 155]	32.5[14.0, 69.0]	51.0[4.00, 155]	
Number of ICI cycles				0.38
1	4 (33.3%)	3 (50.0%)	7(38.9%)	
2	4 (33.3%)	2 (33.3%)	6(33.3%)	
≥3	4 (33.3%)	1 (16.7%)	5(27.8%)	
Mean (SD)	2.75 (2.22)	1.67 (0.82)	2.39(1.91)	
Median [Min, Max]	2.00 [1.00, 8.00]	1.50[1.00, 3.00]	2.00 [1.00, 8.00]	

ICI, immune checkpoint inhibitor; PD, progressive disease; PD-L1, programmed death-ligand 1; PR, partial response, SD, stable disease; PD, progressive disease. Tumour responses were assessed using computer tomography and determined using RECIST version 1.1.

	Mild group (N=12)	Severe group (N=6)	All Cases (N=18)	P
Efficacy of ICIs				0.299
PR	5 (41.7%)	0 (0%)	5 (27.8%)	
SD	6 (50.0%)	4 (66.7%)	10 (55.6%)	
PD	1 (8.3%)	0 (0%)	1 (5.6%)	
Missing	0 (0%)	2 (33.3%)	2 (11.1%)	
ICI, immune checkpoint inhibitor; PD, progressive disease; PD-L1, programmed death-ligand 1; PR, partial response, SD, stable disease; PD, progressive disease. Tumour responses were assessed using computer tomography and determined using RECIST version 1.1.				

The median age among the 18 patients was 60 years old (range from 29.0 to 79.0 years), 83.3% were male, and a history of smoking was reported in 33.3%. Alcohol consumption was reported in 16.7% of the patients. 27.8% of patients had a history of diabetes and 38.9% of patients had hypertension. Lung cancer was the most common cancer diagnosis (55.6%) and thymic carcinoma (22.2%), followed by bladder cancer (5.56%), sigmoid colon cancer (5.56%), head and neck cancer (5.56%) (Figure 1). In addition, the majority of patients (72.2%) were in stage IV cancer at the time of ICI commencement. Half of the patients were asymptomatic. The most common clinical manifestations included dyspnea and fatigue.

In the mild myocarditis group, the mean age was 63.3 years old (range from 45.0 to 79.0 years) and in the severe group was 46.8 years old (range from 29.0 to 67.0 years). Lung cancer was the most prevalent cancer type in the mild group (75.0%), but thymic carcinoma was the most common cancer type in the younger age group (50.0%). Furthermore, none of the patients with severe myocarditis had a history of hypertension.

3.2 Treatment-related Characteristics

The majority of patients (61.1%) received ICIs as first-line therapy, while chemotherapy, not immunotherapy, has been used as first- or second-line therapy in the severe myocarditis group. In 66.7% of patients with severe myocarditis, ICIs have been used as a third-line or later treatment (Table 1). According to the distinct mechanisms of action of ICIs, 15 patients (83.3%) received anti-PD-1 therapy and the remaining 3 patients (16.7%) received anti-PD-L1 treatment. The percentage distribution of 18 cases was as follows: Pembrolizumab 33.3%, Sintilimab 22.2%, Camrelizumab 16.7%, and other ICIs 27.8%, according to various ICI administered.

The median cycles of the onset of immune-related myocarditis from the initiation of ICI infusion were 2 (range, 1-8 cycles), and the median time was 51.0 days (range, 4-155 days). The number of days from the initiation of ICI infusion to the presentation of myocarditis in the mild group was longer than in the severe

group. The mean time was 64.8 and 36.2 days in the mild versus severe group, respectively. Approximately 72.2% of patients developed myocarditis after 1 or 2 ICI infusion cycles. For patients with severe myocarditis, only one patient received more than 3 cycles at the time of diagnosis. Tumour responses were assessed using computer tomography and determined using the RECIST version 1.1. Overall, 5 patients experienced partial responses, 10 patients exhibited stable disease, and 1 patient had progressive disease after ICI administration.

Patients were divided into three groups based on their treatment regimen. The proportion of participants who used ICI alone, chemotherapy + ICI, and targeted therapy + ICI were 50.0%, 44.4%, and 5.60%, respectively. Due to the small number of participants in this study, people treated with ICI alone (N=9) and Chemo+ICI (N=8) will be discussed further (Table 2). The median onset time was earlier in the Chemo + ICI group compared with ICI alone cohort (34.5 v.s 69.0 days). In mild-myocarditis group, the onset time of ICI alone was 109 days, which was longer than patients who received Chemo + ICI (onset time:34.5 days). Timing of the development of immune-related myocarditis with a mean delay of 78.9 days and a median of 2 doses from the first ICI administration in the monotherapy group. Details of other information in each subgroup are listed in the Table 2.

Table 2
Comparison of treatment regimens in myocarditis patients.

	ICI alone (N=9)	ICI+Chemo (N=8)	P value
Age			0.96
Median [Min, Max]	60.0 [33.0, 79.0]	61.5 [29.0, 75.0]	
Cancer type			0.08
lung cancer	3 (33.3%)	7 (87.5%)	
thymic carcinoma	3 (33.3%)	1 (12.5%)	
other cancers	3 (33.3%)	0 (0%)	
Initial ICI treatment line			1.09
1st	6 (66.7%)	5 (62.5%)	
2nd	1 (11.1%)	1 (12.5%)	
≥3rd	1 (11.1%)	2 (25.0%)	
Missing	1 (11.1%)	0 (0%)	
Efficacy of ICIs			0.61
PR	2 (22.2%)	3 (37.5%)	
SD	6 (66.7%)	4 (50.0%)	
PD	0 (0%)	1 (12.5%)	
Missing	1 (11.1%)	0 (0%)	
ICI type			1.00
Anti-PD-1	7 (77.8%)	7 (87.5%)	
Anti-PD-L1	2 (22.2%)	1 (12.5%)	
Number of ICI cycles			0.12
Mean (SD)	3.22 (2.39)	1.63 (0.744)	
Median [Min, Max]	2.00 [1.00, 8.00]	1.50 [1.00, 3.00]	
Times to diagnosis			0.06
Mean (SD)	78.9 (52.0)	33.8 (22.2)	
Median [Min, Max]	69.0 [7.00, 155]	34.5 [4.00, 57.0]	
Chemo: chemotherapy.			

3.3 Laboratory and other relevant examination results

Baseline laboratory results were available in the majority of our patients. Troponin was elevated in all of the patients. Troponin values in most patients did not return to normal after six months and the levels fluctuate considerably during therapy. The median troponin was significantly higher in the severe myocarditis group than patients with mild myocarditis (61.2 ng/L vs. 440 ng/L, respectively, $p=0.0057$). B-type natriuretic peptide (BNP), creatine kinase-MB (CK-MB) and myoglobin in the severe cohort were also higher in comparison to the mild cohort, but the difference was not statistically significant. A significant elevation in lactate dehydrogenase (LDH) was observed between those two groups ($p=0.04$). The median value of LDH was 233 IU/L and 519 IU/L in the mild and severe myocarditis group, respectively. White blood cells (WBC), neutrophils, lymphocytes, eosinophils, and monocytes were higher in the severe group compared with the mild group, but those parameters were not statistically significant (Table 3). CD3, CD8, and CD4 serum levels were also examined prior to ICI therapy, but no significant differences were seen. Blood lipid levels, including total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were also measured in our study. No statistical significance was detected between the severity of myocarditis and blood lipid levels. Higher TC and LDL-C levels and lower HDL-C levels were found in severe group, large-scale studies considering the relationship are needed to further explore.

Table 3
The lab result, cardiac markers and ECG in all patients.

	Overall (N=18)	Mild group (N=12)	Severe group (N=6)	P value
WBC*10⁹/L				0.15
Mean (SD)	5.37 (1.65)	4.94 (1.42)	6.23 (1.87)	
Median [Min, Max]	4.85 [2.52, 8.79]	4.70 [2.52, 7.77]	5.77 [4.53, 8.79]	
Neutrophils*10⁹/L				0.21
Mean (SD)	3.51 (1.86)	3.07 (1.69)	4.38 (2.03)	
Median [Min, Max]	3.20 [1.20, 8.10]	2.81 [1.20, 6.13]	3.39 [2.85, 8.10]	
Lymphocytes*10⁹/L				1.00
Mean (SD)	1.19 (0.70)	1.17 (0.69)	1.22 (0.77)	
Median [Min, Max]	0.91 [0.31, 2.37]	0.90 [0.43, 2.37]	0.99 [0.31, 2.25]	
Eosinophils*10⁹/L				0.37
Mean (SD)	0.15 (0.14)	0.17 (0.16)	0.10 (0.10)	
Median [Min, Max]	1.00[0, 0.48]	1.00 [0.02, 0.48]	0.07 [0, 0.23]	
Monocytes*10⁹/L				0.17
Mean (SD)	0.41 (0.15)	0.37 (0.15)	0.49 (0.12)	
Median [Min, Max]	0.41 [0.06, 0.64]	0.40 [0.06, 0.52]	0.45 [0.36, 0.64]	
LDH (IU/L)				0.04
Mean (SD)	485 (455)	358 (342)	740 (575)	
Median [Min, Max]	260 [144, 1760]	233 [144, 1350]	519 [230, 1760]	
CK-MB (ng/ml)				0.28
Mean (SD)	47.8 (76.0)	26.8 (38.2)	89.7 (115)	
Median [Min, Max]	8.30 [0.89, 300]	7.22 [0.89, 125]	44.5 [1.79, 300]	
Myoglobin (ng/ml)				0.14

CK-MB: Creatine kinase-MB; BNP: B-type natriuretic peptide; CRP:C-reactive protein; ECG: Electrocardiographic;LDH:lactate dehydrogenase; NLR:neutrophil-lymphocyte ratio; TC:total cholesterol;TG:triglycerides;LDL-C:low-density lipoprotein cholesterol; HDL-C:high-density lipoprotein cholesterol.

	Overall (N=18)	Mild group (N=12)	Severe group (N=6)	P value
Mean (SD)	688 (912)	505 (751)	1090 (1160)	
Median [Min, Max]	246 [32.7, 3000]	188 [32.7, 2660]	902[123, 3000]	
Missing	2 (11.1%)	1 (8.3%)	1 (16.7%)	
Troponin (ng/L)				0.0057
Mean (SD)	359 (554)	134 (195)	810 (772)	
Median [Min, Max]	111 [22.5, 1990]	61.2 [22.5, 715]	440 [130, 1990]	
BNP (ng/L)				0.34
Mean (SD)	2510 (6980)	774 (1340)	5700 (11600)	
Median [Min, Max]	312[19.0, 29200]	212 [19.0, 4620]	933 [85.0, 29200]	
Missing	1 (5.6%)	1 (8.3%)	0 (0%)	
PCT (ng/ml)				0.87
Mean (SD)	1.07 (1.54)	0.78 (0.960)	1.48 (2.18)	
Median [Min, Max]	0.18[0.02, 5.02]	0.22 [0.02, 2.12]	0.13 [0.03, 5.02]	
Missing	6 (33.3%)	5 (41.7%)	1 (16.7%)	
IL-6 (pg/ml)				0.92
Mean (SD)	265 (805)	401 (1010)	26.3 (32.4)	
Median [Min, Max]	12.8 [2.29, 2690]	12.8 [2.29, 2690]	12.6 [2.77, 77.1]	
Missing	7 (38.9%)	5 (41.7%)	2 (33.3%)	
CRP (mg/L)				0.74
Mean (SD)	45.6 (42.4)	53.1 (49.5)	34.9 (32.0)	
Median [Min, Max]	42.7 [2.97, 131]	68.5 [2.97, 131]	22.2 [4.75, 74.9]	
Missing	6 (33.3%)	5 (41.7%)	1 (16.7%)	
NLR				0.54
Mean (SD)	5.00 (6.05)	4.10 (3.56)	6.81 (9.51)	

CK-MB: Creatine kinase-MB; BNP: B-type natriuretic peptide; CRP:C-reactive protein; ECG: Electrocardiographic;LDH:lactate dehydrogenase; NLR:neutrophil-lymphocyte ratio; TC:total cholesterol;TG:triglycerides;LDL-C:low-density lipoprotein cholesterol; HDL-C:high-density lipoprotein cholesterol.

	Overall (N=18)	Mild group (N=12)	Severe group (N=6)	P value
Median [Min, Max]	3.04 [0.51, 26.1]	2.71 [0.51, 10.6]	3.09 [1.59, 26.1]	
CD3				0.76
Mean (SD)	64.3 (16.0)	66.0 (19.2)	62.0 (11.6)	
Median [Min, Max]	63.6 [39.6, 90.1]	64.7 [39.6, 90.1]	62.4 [46.6, 76.7]	
CD4				0.53
Mean (SD)	28.7 (12.1)	31.7 (14.3)	24.5 (7.6)	
Median [Min, Max]	27.1 [16.1, 58.3]	29.5 [16.1, 58.3]	22.1 [17.1, 35.8]	
CD8				0.64
Mean (SD)	31.3 (15.2)	30.6 (17.7)	32.2 (12.9)	
Median [Min, Max]	27.5 [17.0, 68.6]	27.4 [17.0, 68.6]	31.3 [17.7, 49.4]	
Blood lipid levels				
TC(mmol/L)	4.44 (0.876)	4.39 (1.02)	4.53 (0.569)	0.49
TG(mmol/L)	1.73 (0.702)	1.77(0.744)	1.66 (0.671)	0.82
HDL-C(mmol/L)	1.08 (0.302)	1.19 (0.227)	0.857 (0.328)	0.06
LDL-C(mmol/L)	2.69 (0.783)	2.52 (0.904)	3.02 (0.302)	0.06
ECG				
abnormal T wave	7/18	4/12	3/6	0.63
abnormal ST segment	3/18	3/12	0/6	0.52
conduction defects	3/18	2/12	1/6	1.00
Sinus tachycardia	3/18	2/12	1/6	1.00
Normal ECG	1/18	1/12	0/6	-
CK-MB: Creatine kinase-MB; BNP: B-type natriuretic peptide; CRP:C-reactive protein; ECG: Electrocardiographic;LDH:lactate dehydrogenase; NLR:neutrophil-lymphocyte ratio; TC:total cholesterol;TG:triglycerides;LDL-C:low-density lipoprotein cholesterol; HDL-C:high-density lipoprotein cholesterol.				

Every patient received an electrocardiographic (ECG) examination, and nearly every case of myocarditis showed an irregular ECG (94.4%). It was found that 7 cases had abnormal T waves, 3 cases had an abnormal ST segment, and conduction defects were also found in 3 patients. Additionally, sinus

tachycardia showed in three cases. But such difference in ECG between those two groups did not have statistically significant.

Echocardiography was performed in 17 patients. Two patients were discovered to have a ventricular wall movement disorder, and two patients had left ventricular systolic dysfunction ($LVEF \leq 50\%$). One patient was diagnosed with severe myocarditis and third-degree atrioventricular block, with an LVEF of 40%. Coronary angiography was also performed in this patient which revealed the anterior descending branch, circumflex branch and right coronary artery were stenosed and the first diagonal branch had the most severe stenosis of nearly 40%. However, the patient had no history of coronary heart disease. Coronary angiography results were also obtained from the other four patients. One patient in the severe group had stenosis in the left anterior descending branch, circumflex branch and right coronary artery. The diagnosis of myocarditis in these 2 cases was made by a multidisciplinary team and they all matched with the inclusion criteria. Nevertheless, there are no meaningful findings in the other three patients in the mild group. A cardiac magnetic resonance imaging (MRI) investigation was performed in six cases, however, we were unable to find meaningful data in five of them. Only one patient showed myocardial edema on T2-weighted imaging. Furthermore, one patient with severe myocarditis received cardiac 18F-fluorodeoxyglucose (18F-FDG) position emission tomography magnetic resonance imaging (PET-MRI) which revealed enhanced FDG metabolism at the left ventricle. All patients were treated with steroids once myocarditis was diagnosed. The initial steroid dose was an equivalent of methylprednisolone (1-2 mg/kg), which was sustained for 3–5 days and followed by a long-term oral steroid taper. In four patients, intravenous gammaglobulin was used to relieve their symptoms. Unfortunately, one patient died as a result of myocarditis-related complications.

3.4 Concomitant irAEs and outcomes

66.7% of patients (12 cases) were concurrent with at least one irAE (Figure 2A). 9 patients developed another irAE: six patients were diagnosed with immune-related myositis, two acquired hypothyroidism and one suffered pneumonitis. One patient developed other two irAEs and two patients were concurrent with three irAEs. Overall, there was no difference in the prevalence between the mild and severe groups ($P>0.5$). Myositis ($n=8$) and hypothyroidism ($n=4$) were the most common irAEs in our cohorts.

The difference between those who had additional irAE and those who did not was also discussed in our research (Table 4). Patients without concomitant irAEs ($N=6$) were all males and treated with PD-1 antibodies. The onset time of patients with single irAE was later than patients with multi-irAEs. Baseline characteristics were explored and identified that the alcohol history was significantly different. All patients with concomitant irAEs did not have an alcohol history ($p=0.03$). However, this result should be validated in a larger cohort study. There were also statistically significant changes in LDH and myoglobin. Patients with multi-irAEs have a higher levels of LDH (250 IU/L v.s 603 IU/L, $P=0.02$) and myoglobin (112 ng/ml v.s 950 ng/ml, $P=0.02$). There was also a trend of higher CK-MB, BNP, and a lower troponin in patients with multi-irAEs, although these changes were not statistically significant.

Table 4
Comparison of myocarditis patients with Single irAE or Multi-irAE.

	Single irAE (N=6)	Multi-irAE(N=12)	P value
Severity			1
mild	4 (66.7%)	8 (66.7%)	
Severe	2 (33.3%)	4 (33.3%)	
Age	65.7 (10.5)	53.8 (16.0)	0.12
Gender			0.52
Male	6 (100%)	9 (75.0%)	
Female	0 (0.00%)	3 (25.0%)	
Cancer type			0.42
Other cancers	2 (33.3%)	2 (16.7%)	
lung cancer	4 (66.7%)	6 (50.0%)	
thymic carcinoma	0 (0.00%)	4 (33.3%)	
ICI type			0.52
Anti-PD-1	6 (100%)	9 (75.0%)	
Anti-PD-L1	0 (0.00%)	3 (25.0%)	
Number of ICI cycles	3.33 (2.50)	1.92 (1.44)	0.12
Times to onset	72.0 (50.7)	46.8 (42.8)	0.28
WBC*10⁹/L	4.86 (0.99)	5.63 (1.89)	0.28
Neutrophils*10⁹/L	3.26 (0.70)	3.64 (2.25)	0.74
Lymphocytes*10⁹/L	1.07 (0.65)	1.24 (0.74)	0.85
Monocytes*10⁹/L	0.40 (0.26)	0.42 (0.06)	0.48
Eosinophils*10⁹/L	0.11 (0.09)	0.17 (0.16)	0.74
NLR	4.25 (3.00)	5.38 (7.21)	0.67
CK-MB (ng/ml)	8.60 (10.4)	67.3 (87.3)	0.08
Myoglobin (ng/ml)	112 (65.0)	950 (1003)	0.02

*Data are shown as means±SD. CK-MB: Creatine kinase-MB; BNP: B-type natriuretic peptide; CRP:C-reactive protein; ECG: Electrocardiographic; LDH: lactate dehydrogenase; NLR: neutrophil-lymphocyte ratio.

	Single irAE (N=6)	Multi-irAE(N=12)	P value
Troponin (ng/L)	479 (763)	299 (445)	0.89
BNP (ng/L)	992 (1042)	3341 (8679)	0.72
PCT (ng/ml)	2.45 (2.42)	0.61 (0.91)	0.12
CRP (mg/L)	30.6 (42.1)	50.5 (43.7)	0.64
LDH (IU/L)	250 (107)	603 (519)	0.02
IL-6 (pg/ml)	1351 (1893)	23.2 (30.1)	0.34
CD3	65.3 (17.6)	64.0 (16.5)	1.00
CD4	40.0 (16.6)	24.9 (8.26)	0.15
CD8	22.2 (7.91)	34.3 (16.2)	0.21
*Data are shown as means±SD. CK-MB: Creatine kinase-MB; BNP: B-type natriuretic peptide; CRP:C-reactive protein; ECG: Electrocardiographic; LDH: lactate dehydrogenase; NLR: neutrophil-lymphocyte ratio.			

3.5 Comparison of the baseline levels of serum myocardial enzymes and LDH between different groups.

Our results revealed that the serum myocardial enzymes and LDH may play an suggestive role in the early diagnosis of immune-related myocardial injury. But there was no statistical difference of baseline levels of those markers between patients with severe myocarditis and mild myocarditis ($P > 0.05$, Table 5). To further exploring the difference of those marker between patients with and without myocarditis, we retrospectively screened 30 patients without myocarditis in our hospital who treated with ICIs. There was no statistical significance on the differences in serum myocardial enzymes and LDH between patients who experienced immune-related myocarditis group and who did not experienced ($P > 0.05$, Figure 2B). We also analyzed the changes of those markers during treated with ICIs in patients without immune-associated myocarditis. The comparison of LDH, troponin, CK-MB, BNP and myoglobin levels between the baseline levels and after received one or two cycles of ICIs were not statistically significant ($P > 0.05$).

Table 5
Comparison of the baseline levels of serum myocardial enzymes and LDH between different groups

	Mild group	Severe group	P value
Troponin (ng/L)	12.9 (8.52)	8.58 (3.79)	0.67
Myoglobin (ng/ml)	31.3 (13.2)	24.1 (3.61)	0.71
CK-MB (ng/ml)	1.49 (1.12)	1.54 (0.546)	0.57
BNP (ng/L)	94.5 (57.5)	59.0 (43.8)	0.67
LDH (IU/L)	221 (99.5)	179 (29.2)	0.34

3.6 The role of troponin and LDH levels in diagnosing severe myocarditis.

To confirm our findings, we searched PubMed for relevant articles and selected 30 for analysis.. The results are coincident with ours. Patients with severe myocarditis have a higher troponin ($p=0.005$) and an earlier onset time (19.1 v.s 29.7 days, $p=0.014$) compared with the mild group. In addition, significant difference was found in the outcome between two groups (Table 6). Patients in the severe group have a higher mortality (53.3% v.s 6.7%, $p=0.041$). The LDH in most studies were not eligible and thus we did not analyzed.

Table 6
Patients with immune-related myocarditis searched in Pubmed were analysed.

	Mild group (N=15)	Severe group (N=15)	P value
Number of ICI cycles			0.64
Mean (SD)	1.53 (1.06)	1.27 (0.458)	
Median [Min, Max]	1.00 [1.00, 5.00]	1.00 [1.00, 2.00]	
Times to diagnosis			0.041
Mean (SD)	29.7 (20.8)	19.1 (9.38)	
Median [Min, Max]	28.0 [4.00, 90.0]	19.0 [4.00, 45.0]	
Troponin (ng/L)			0.005
Mean (SD)	1200 (1280)	4540 (4580)	
Median [Min, Max]	1030 [44.0, 3720]	3230 [806, 16300]	
Outcomes			0.014
Lived	14 (93.3%)	7 (46.7%)	
Died	1 (6.7%)	8 (53.3%)	

4. Discussion

Immune checkpoint inhibitors have drastically improved clinical outcomes and they are increasingly being licensed for use in the early stage of cancer and combination with other anti-tumor therapies such as chemotherapy or targeted therapy ¹. However, due to the increased usage of these medications, immune-related adverse events in patients receiving ICIs are becoming more widely recognized, which may limit their clinical applicability. One of the most fatal irAEs is immune-related myocarditis. Due to its severity and high mortality, there is a growing interest in the further study⁹. The risk of myocarditis varies depending on the treatment regimen and the ICI drugs used. Overall, ICI-related myocarditis was reported to be around 1% of the incidence. Previous research had shown that dual ICIs regimens caused a higher incidence than monotherapy ¹⁰. The incidence of monotherapy, dual ICIs therapies and ICI plus chemotherapy were all found to be 3.1%, 5.8%, and 3.7%, respectively. There were also variations in the occurrence of myocarditis among the various classes of ICIs ⁸. Myocarditis is more likely to be caused by the CTLA-4 antibody. Furthermore, it has been observed that nivolumab (anti-PD-1) had a decreased incidence of cardiac irAEs ¹¹. The underlying mechanism still unclear. T-cell infiltration into the myocardium, increased auto-antibodies acting on self-antigens, and increased T cells reacting to antigens shared by cancer and normal cells could all be contributors to the pathomechanism of the irAEs ¹².

There is a scarcity of information on this potentially fatal adverse event. Our current understanding of the illness is limited, and patient-derived evidence is scarce. Recent research has concentrated on examining the disease's overall characteristics while neglecting the individual variabilities. The goal of this retrospective study was to provide new insights about such a rare irAE and explore risk factors for severe myocarditis and multi-irAEs. According to Common Terminology Criteria for Adverse Events (CTCAE), AE grading is currently based on the outcomes of biomarkers, ECG, symptoms and cardiac complications¹³. Patients with grade 1 need to be closely monitored during therapy. The patient in grade 1 is asymptomatic but has aberrant cardiac biomarkers with an irregular ECG. Compared to grade 1, patients with grade 2 have minor symptoms. Symptoms in grade 3 patients are more severe and required the use of steroids to manage. Grade 4 is defined as moderate to severe decompensation of life-threatening situations that demand intravenous injection of medication or intervention. In our study, patients were divided into groups based on their clinical manifestations and whether they had haemodynamic complications such as heart failure, cardiogenic shock, or arrhythmia, which may be more useful in assisting clinicians in determining a patient's condition and prognosis. Patients in severe group were categorized as grade 3–4 toxicity and in the mild group were classified as grade 1–2 toxicity.

It is of critical importance to distinguish between mild and severe myocarditis due to the high mortality rate. Several studies have identified treatment regimens, comorbidities such as hypertension and diabetes, and tobacco use as risk factors for cardiotoxicity¹⁴. However, Subgroup analyses based on age, gender, tobacco usage, diabetes, and cancer stage revealed no differences in our study. According to our findings, hypertension and cancer type may not correlate to the severity of myocarditis. Contrary to earlier reports, our data have shown that none of the patients in the severe group had hypertension. In addition, none of the 18 patients had coronary artery disease or any other type of cardiac disease. No statistical significance was detected between the severity of myocarditis and blood lipid levels.

The most common malignancy type in the mild group was lung cancer, while the most common type in the severe group was thymic carcinoma. The fundamental cause of the discrepancy in our study was unknown. This could be due to the fact that a large percentage of patients with thymic cancer have an autoimmune syndrome, which could be an independent risk factor for ICI-associated cardiotoxicity. The thymus plays an essential role in the development of T-cells. Although autoimmune disease patients are not the focus of our research, the function and composition of T-cells, which are important components of the immune system, are similarly aberrant in the thymoma microenvironment¹⁵. Therefore, it is critical to keep track of the occurrence of myocarditis in thymic carcinoma patients receiving ICI.

According to the literature, immune-related myocarditis frequently develops soon after starting ICI therapy. The median time of onset was 34 days, with the majority of cases occurring within three months⁸. In our studies, the median length was 2 cycles and the onset time was 51 days (range: 4-155), which is longer than previous reports¹⁶. Patients with severe myocarditis had a faster onset time than those with mild myocarditis. Myocarditis is more likely to present at an early age in patients in the mild group who were treated with combination therapy, although the results of onset time were not statistically

significant. Treatment lines, ICI type, number of ICI cycles, and ICI efficacy all had no statistically significant differences. Recent research suggested that irAEs have been linked to a long-term response and therapeutic benefit^{16–18}. Among the 16 patients, 5 patients had a partial response (PR), 10 patients had stable disease (SD), and 1 patient had progressive disease (PD).

Early identification of severe myocarditis followed by timely treatment is essential to reduce mortality and assisting physicians in personalized medicine decision-making. Our study suggested that the troponin and LDH might contribute to recognize the severe myocarditis as soon as possible. Previous studies have demonstrated that monitoring troponin during treatment is reasonable^{6, 19, 20}, but the relationship between LDH and cardiotoxicity were never reported. Patients with severe myocarditis are more likely have a higher LDH. Furthermore, real-time screening of concomitant irAE is essential in patient experiencing immune-related myocarditis, especially in patient with higher LDH and myoglobin levels. In addition, the level of troponin also correlated with outcomes.

The diagnosis and management of this disorder continue to be a clinical and research challenge. Myocardial biopsy is the gold standard for diagnosis. However, the complications may have an adverse effect on clinical utility and outcomes²¹. The relevance of cardiac MRI, FDG-PET, and coronary angiography in the identification of myocarditis has also been highlighted in recent research^{22–24}. However, performing those examinations may miss the optimal treatment time and result in negative repercussions. A multidisciplinary team (MDT) approach is crucial for assessing suspected immune-related myocarditis, since it aids decision-making and lowers death rates. The mortality rate was reported to be as high as 46%²⁵, while in our study was 5.6% (1/18). Early identification by MDT and the use of steroids may have contributed to the lower mortality rate. For severe myocarditis, there is no question that corticosteroids should be used, but the treatment of mild myocarditis by steroids is still unknown and requires further research. Our study provides clinical relevance and rationale for initiating corticosteroid therapy regardless of the severity of myocarditis. All 18 patients in our study received steroid treatment. An equivalent dose of (methyl)prednisolone (1-2 mg/kg) is given for the first 3–5 days, followed by a long-term oral steroid taper. In addition to the use of steroids, intravenous gammaglobulin should be considered to alleviate the symptoms of patients based on clinical need. After being diagnosed with myocarditis, no one disputes ICIs again.

Our research fills a gap in the literature and sheds new light on a rare irAE. However, there are a few flaws worth mentioning. It's worth noting the inherent bias in any single-institution retrospective analysis, and also the limited sample population in our study. Due to the challenges in diagnosis, the vast majority of myocarditis events may be overlooked or misdiagnosed. Furthermore, extreme vigilance is essential in the therapy and the avoidance of excessive corticosteroid usage for bias results. Further investigation is required as an outcome of our findings.

Declarations

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Conflict of Interest Statement

All authors declare no conflicts of interest in relation to this work.

Data availability statement

All data relevant to the study are available from the corresponding author on reasonable request.

Author contributions

ML: Conceptualization, Supervision and Data curation; YL: Writing original draft, Formal analysis, and Validation; Other authors: Supervision and Validation

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Figures

Figure 1

Cancer type distribution of patients with mild or severe Myocarditis.

Figure 2

A: The distribution of concurrent irAEs in our study. B: Serum myocardial enzymes and LDH between patients with or without immune-related myocarditis.