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Case Report Open Access

Histopathological Characteristics in Autoimmune Myocarditis Associated with Pembrolizumab: A Case Report and Literature Review

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Abstract

Introduction: Immune checkpoint inhibitors (ICIs) are revolutionizing cancer therapy and have significantly improved the clinical outcome of multiple tumor types. ICIs-induced myocarditis is a rare, but potential fatal side effect that requires prompt treatment. The histopathological features of ICIs-induced myocarditis are not well characterized due to limited number of cardiac biopsy or autopsy performed.

Case presentation: Here we reported an autopsy finding from a 70-year-old white male with severe myocarditis induced by pembrolizumab administered during the treatment for high grade urothelial carcinoma. He initially presented with left eye ptosis, diplopia, fatigue, and shortness of breath for four days, followed by quickly developed brachycardia and complete heart block. He was treated with high-dose glucocorticoids, but his condition continued to deteriorate. He experienced two cardiopulmonary arrests and died on the fifth day of admission. Sections from heart show extensive myocarditis with dense mixed inflammatory infiltration and patchy myocyte necrosis. Focal endothelitis associated with scattered micro-hemorrhages are also identified.

Discussion and conclusion: We reviewed the published case reports or case series from 2015 to 2018 and identified 13 cases of ICIs-induced myocarditis with available histopathologic description. We summarized the finding of these 14 patients including our own case in this paper. Although lymphocytic myocarditis was present in all cases, some histopathologic features are not consistently observed. Better characterization of histopathologic features of ICIs-induced myocarditis will help us understand the mechanism of this fatal toxicity and lead to potentially more specific treatment.

Keywords: Immune checkpoint inhibitors; Immune-related adverse events; ICI-induced myocarditis; Histopathological characteristics; Autopsy

Introduction

Immune checkpoint inhibitors (ICIs) have significantly improved clinical outcomes in numerous solid and liquid malignancies, which have led to rapid incorporation of these agents in both community and academic practice in the United States and abroad. Because of the rapidly growing list of clinical indications for ICIs, familiarity with the immune-related adverse events (irAEs) that ICIs induce is also critical to the safe use of these agents.

Myocarditis is one of the most serious and fatal side effects associated with ICI therapy. Recently published case series and reviews have focused on the clinical presentation and radiological and laboratory findings, with the intention to highlight clinical management and outcomes of patients with cardiotoxicity induced by ICI [1-3]. However, the histopathological changes of ICI-induced myocarditis have not been well characterized, partly because of the limited number of cases with available cardiac biopsy or autopsy results. Here, we report autopsy findings for a patient with severe myocarditis induced by pembrolizumab (anti-programmed cell death 1 [PD-1]) administered during treatment for urothelial carcinoma, and we review the histopathological features of 14 cases of myocarditis: our own case, and 13 reported in the literature from 2015 to 2018.

Case Presentation

The patient was a 70-year-old white man with a past medical history notable for hypertension, hyperlipidemia, former smoking (45 pack years), Agent Orange exposure, diminished bilateral hearing, gout, and erectile dysfunction. He had also developed gross hematuria with associated low back pain; those symptoms prompted a computed tomography (CT) scan of the abdomen/pelvis that revealed a bulky bladder mass, enlarged pelvic lymphadenopathy, and left-sided hydroureteronephrosis. The patient underwent two cystoscopies with transurethral resection of bladder tumor; these revealed evidence of a high-grade papillary urothelial cancer of the bladder invading into the muscularis propria. The patient also underwent a lymph node biopsy, the findings of which were consistent with metastatic urothelial cancer. A staging positron emission tomography CT scan identified the pelvic lymphadenopathy and a bone metastasis involving the patient's right acetabulum. On evaluation, the patient appeared fit and his Eastern Cooperative Oncology Group performance status was 1. He underwent a percutaneous nephrostomy tube placement and received six cycles of chemotherapy with cisplatin (50 mg/m^2), gemcitabine (900 mg/m^2), and ifosfamide (1000 mg). Post-treatment imaging studies showed a partial response in his bladder and lymph nodes and sclerotic changes in his bone metastases.

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A detailed discussion about the next treatment options was undertaken and the patient decided to begin treatment with pembrolizumab. He received a standard, 200 mg, intravenous dose. Fifteen days after the initial dose, he presented to the emergency department with left eye ptosis, diplopia, fatigue, and shortness of breath over a 4-day duration. An initial electrocardiogram (ECG) revealed sinus bradycardia at 51 beats per minute with profound prolongation of the PR interval to 480 msec and slight widening of the QRS complexes, but no ischemic changes. An ECG 4 months prior had revealed a PR interval of 166 msec. On laboratory analysis, the patient had an elevated level of troponin T (2,740 ng/L; reference range, ≤ 18 ng/L), creatine kinase (CK) (12,706 U/L; reference range, 20-200 U/L), CK muscle/brain (CK-MB) (272.3 ng/mL; reference range, ≤ 104 ng/mL), and serum creatinine (1.95 mg/dL; baseline for patient, 1.5 mg/dL). He also had elevated liver transaminases: his alanine transaminase level was 240 U/L (reference range, <41 U/L) and his aspartate transaminase level was 591 U/L (reference range, ≤ 40 U/L). The patient's complete blood count was notable for mild leukocytosis. He had a white blood cell count of 13.8 K/uL with left shift and an absolute neutrophil count of 12.1 K/uL, and he had lymphopenia with an absolute lymphocyte count of 0.88 K/uL. Serum studies for human immunodeficiency virus, hepatitis B, and hepatitis C were all negative. A cytomegalovirus (CMV) Ab IgG test was positive. The patient's blood culture was negative for microorganisms, but his urine culture was positive for *Klebsiella oxytoca* and *Pseudomonas aeruginosa.*

The patient was admitted to the intensive care unit and given broad-spectrum antibiotics and methylprednisolone (1 mg/kg every 12 hours) for suspected immunotherapy-mediated myositis. About 7 hours after admission, the patient's heart rate dropped to 30-40 beats per minute and a second ECG showed intermittent third-degree heart block. A temporary pacemaker was inserted immediately and the patient was also intubated. An initial echocardiogram revealed normal left ventricular systolic function with a left ventricular ejection fraction (LVEF) of 68% and normal wall motion. The patient received one cycle of plasmapheresis, but the treatment was discontinued due to severe hypotension. He rapidly developed severe lactic acidosis, acute kidney injury, and worsening acute hepatic injury. He was started on continuous veno-venous hemofiltration and required vasopressor support.

Two days after admission, a repeat echocardiogram showed mildly to moderately depressed left ventricular systolic function with an LVEF of 43% and global hypokinesis. In addition, the repeat echocardiogram showed a minimal pericardial effusion that had not been present on the initial echocardiogram. Given the severity of his condition, the patient did not undergo an endomyocardium biopsy and instead underwent a muscle biopsy of his right quadriceps on day 3 of admission. The biopsy revealed focal muscle fiber necrosis and mild lymphocyte infiltration. On day 4 of admission, a T-SPOT. TB test was negative for tuberculosis and the patient was given a dose of infliximab. Over the 5 days that the patient was hospitalized, his CK level dropped (admission day 0, 12,706 U/L; day 1, 4,204 U/L; day 2, 1,941 U/L; day 3, 1,077 U/L; day 4, 872 U/L; and day 5, 584 U/L), but his troponin T level climbed (admission day 0, 2,740 ng/L; day 1, 3,694 ng/L; day 2, 4,076 ng/L; day 3, 6,495 ng/L; day 4, 7,008 ng/L; and day 5, 11,070 ng/L). The patient's condition continued to deteriorate on day 5. He experienced two cardiopulmonary arrests and died.

A broad panel of immune-related antibody tests were ordered during admission. The pertinent positive findings included an elevated acetylcholine receptor binding antibody (Ab) level of 1.2 nmol/L (reference range, ≤ 0.02 nmol/L), an antinuclear antibody homogenous pattern (titer, 1:320), and a positive finding of smooth muscle Ab (titer, 1:20). A cytokine 12 send-out panel did not reveal any grossly elevated cytokines.

Characteristics	No. (N=14)
Age, years	23 to 80 (average, 64.7)
Sex	
Male	9
Female	5
Cardiovascular risk factors	
MI and/or PVD	3
Hypertension	5
None	3
Unknown	3
Cancer type	
Melanoma	8
Lung adenocarcinoma	2
Urothelial carcinoma	1
Endometrial carcinoma	1
Renal cell carcinoma	1
CMML	1
Immunotherapy type	
Anti-PD-1 or PD-L1	6
Anti-CTLA-4	4
Combined	3
Anti-CTLA-4 with other	1
Onset of symptoms	
First dose	6
Second dose	3
Third dose or later	5
Concomitant side effects	
Neuromuscular side effects	5
Thyroiditis	2
Hepatitis	2
Colitis	1
Cardiac biomarkers	
Elevated troponin	11
Elevated CK-MB	7
Clinical outcomes	
Death from myocarditis	9
Death from primary cancer	1
Recovery	4

Table 1: Clinical information from 14 cases of biopsy- or autopsy-confirmed ICI-induced myocarditis.

Characteristics	No. (%) (N=14)
Specimen type	
Cardiac biopsy	7 (50)
Autopsy	7 (50)
Histopathologic findings	
Lymphocytic or lymphohistiocytic infiltration	14 (100)
Eosinophils	5 (36)
Giant cells	2 (14)
Plasma cells	2 (14)
Neutrophils	1 (7)
Mast cells	1 (7)
Myocyte necrosis	6 (43)
Interstitial fibrosis	4 (29)
IHC findings	
CD3+ T lymphocytes (predominantly CD8+ T cells)	6 (43)
Elevated PD-L1 staining in inflamed cardiomyocytes	3 (21)

Table 2: Histopathological findings from 14 cases of ICI-induced myocarditis.

The patient's family agreed to an autopsy, which was performed one day after the patient's death. The heart weighed 630 grams and had a smooth pericardium and epicardium. The left ventricle had mild concentric hypertrophy with a thickness of 2.2 cm. There were two ill-defined, pale areas in the left anterior and posterior ventricular wall adjacent to the septum (Figure 1A). Severe calcific atherosclerosis of the left anterior descending artery and mild atherosclerosis of the left circumflex artery were identified.

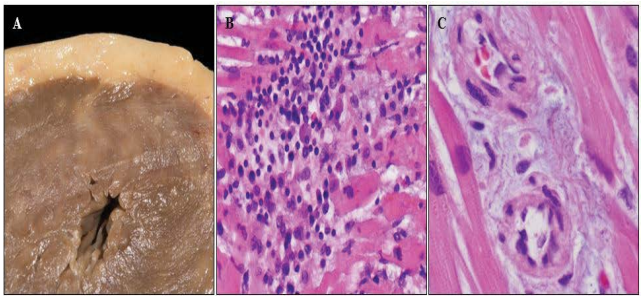

Figure 1: Gross finding and histopathologic features of ICI-induced myocarditis **(A)** Gross picture of heart showing an ill-defined pale areas in the left anterior wall **(B)** Mixed inflammatory infiltration of myocardium (lymphocytes, plasma cells, eosinophils and mast cells) and myocyte necrosis **(C)** Endothelitis with infiltrating lymphocytes and injured endothelial cells.

Hematoxylin-and-eosin-stained sections from the heart revealed diffuse myocarditis with marked inflammatory infiltration—consisting predominantly of lymphocytes admixed with histiocytes, plasma cells, scattered eosinophils, and mast cells—within the myocardium (Figure 1B). Patchy myocyte necrosis and edema were present, as were hypertrophic reactive myocytes. Focal endothelitis was noted in the inflamed myocardial tissue and was associated with scattered micro-hemorrhages (Figure 1C). No neutrophils or viral inclusions were identified, and immunohistochemical staining for CMV was negative. The majority of the infiltrated lymphocytes were CD8+ T lymphocytes, and there was also a minor population of CD4+ T lymphocytes (Figures 2A and 2B). Only scattered CD20+ B lymphocytes were present. CD68 and CD163 highlight markedly increased histiocytes (Figure 2C). No granulomas were identified. Programmed death receptor ligand 1 (PD-L1) staining was positive in the areas of myocytes associated with dense

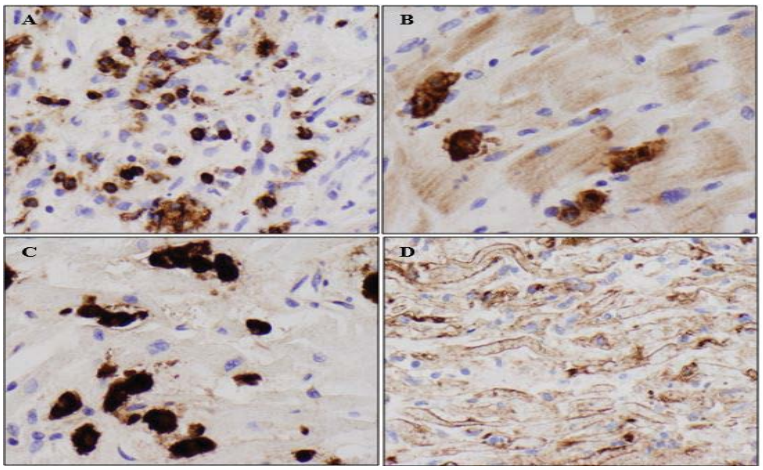

Figure 2: IHC staining of inflamed myocytes **(A)** CD8 staining demonstrated that the majority of T-lymphocytes were CD8+ T-cells **(B)** CD4 staining highlighted small percentage of CD4+ T-lymphocytes **(C)** CD68 stained histiocytes (**D)** Increased membranous staining of PD-L1 in the inflamed and damaged myocytes.

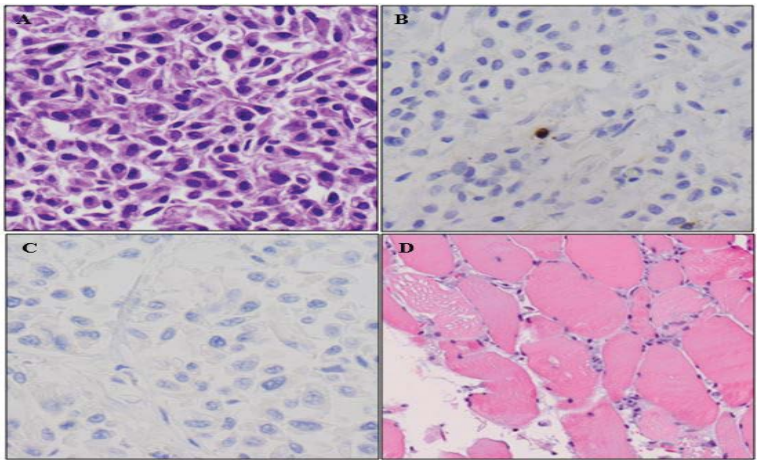

Figure 3: Histopathologic findings of urothelial carcinoma of bladder and skeletal muscle biopsy **(A)** High grade urothelial carcinoma of bladder with minimal treatment effect **(B)** Rare lymphocyte in bladder tumor was highlighted by CD4 **(C)** IHC for PD-L1 was negative in tumor cells **(D)** Skeletal muscle biopsy showed mild lymphocytic infiltration and damaged myocytes.

inflammatory infiltration (Figure 2D). The smooth muscle of the vessels adjacent to the heart was free of lymphocyte infiltrates and negative for PD-L1. Trichome staining demonstrated focal early fibrosis that was reflective of the recent myocyte injury.

The tissue sections from the bladder showed an invasive, high-grade urothelial carcinoma with small foci of necrosis (Figure 3A). The tumor had invaded through the muscularis propria and into the perivesicular adipose tissue. Only scanty lymphocytic infiltration in the peritumor area, consisting mainly of CD4+ T lymphocytes, was seen (Figure 3B). No intra-tumor lymphocytes were present. PD-L1 staining was negative in the tumor cells (Figures 3C and 3D).

Discussion

ICI-associated cardiotoxicity is a serious irAE because it can lead to potentially fatal myocarditis. The incidence of ICI-related myocarditis is up to 1.14% in patients receiving ICI treatment [2]. A recent literature review identified 99 cases of cardiotoxicity induced by cancer immunotherapy in 73 studies published from 1996 to 2017; 45% of the patients presented with myocarditis. The overall mortality rate was 35% [3]. A retrospective analysis of VigiLyze, a World Health Organization pharmacovigilance database, revealed 614 fatal toxic events, of which myocarditis had the highest fatality rate (39.7%) for irAEs [4]. A recent cohort from eight center registries reported 35 patients with immunotherapy-associated myocarditis, nearly 50% of whom experienced major adverse cardiac events, including six cases of cardiovascular death [2]. Cardiotoxicity is more commonly seen in patients treated with combined anti-PD-L1 with anti-cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), but it has also been reported in patients treated with monotherapy [4].

Most published literature emphasizes the clinical presentation, management, and outcomes of ICI-associated myocarditis. Only a few case reports describe the histopathological features of this potentially fatal event. After reviewing published case reports and case series published from 2015 to 2018, we identified 13 cases with histopathological descriptions of biopsy- or autopsy-confirmed myocarditis induced by ICI therapy [3,5-12]. Thus, including our own patient, we had data for 14 patients—nine men and five women—with an average age of 64.7 years (range, 23 to 80 years) (Table 1). Melanoma was the most common cancer type (eight patients), and the remaining patients had lung adenocarcinoma (two patients), urothelial carcinoma (one patient), endometrial carcinoma (one patient), renal cell carcinoma (one patient), or chronic myelomonocytic leukemia (one patient). Ten patients received monotherapy (six received anti-PD-1 or PD-L1, and four received anti-CTLA-4), and three patients were treated with combined therapy. One 23-year-old male melanoma patient was treated with ipilimumab and vemurafenib (a BRAF inhibitor). Most patients had symptoms after receiving the first or second dose of treatment (six patients after the 1st dose and three patients after the 2nd dose). Five patients had concomitant neuromuscular side effects, including double vision, neck weakness, difficulty in ambulation, or imbalance, and four of these patients, including our patient, had a skeletal muscle biopsy that confirmed lymphocytic myositis with myocyte damage [5,8]. This phenomenon has been observed in other large series, in which about 25% of patients with ICI-associated myocarditis had concomitant myositis and 10-11% had myasthenia gravis [13,14]. Eleven of the 14 patients had elevated troponin levels, and half of the patients had increased CK-MB levels. Nine of 14 patients died of myocarditis, and eight out of these patients had onset of symptoms after receiving their first or second doses of immunotherapy. This finding suggests that early-onset myocarditis tends to be fulminant and fatal.

Seven of the patients underwent cardiac biopsy, and in the other seven cardiac specimens were collected during autopsy. The gross findings of the heart in five of the autopsy cases were non-specific and included cardiomegaly, left ventricular hypertrophy, mottling/discoloration of the myocardium, focal fibrosis, and gelatinous changes [3,7,11]. The histopathological findings from the 14 cases of proven ICI-induced myocarditis are summarized in Table 2. Microscopically, all cases showed lymphocytic or lymphohistiocytic myocarditis, and myocyte necrosis was observed in six cases. In six cases, immunohistochemical staining confirmed that the infiltration is predominantlyCD8+ T lymphocytes. Five cases, including our case, also involved eosinophilic infiltration, and only two cases had giant cells identified. Infiltration with other inflammatory cells, including plasma cells, mast cells, and neutrophils, was also reported. Interstitial fibrosis was also observed in four cases. Interestingly, our case is the first report of focal endothelitis and microscopic hemorrhage, indicating endothelial cell damage. Three cases of fulminant myocarditis, ours and two others, demonstrated increased membranous staining of PD-L1 in injured cardiomyocytes [8]. Upregulation of PD-L1 is observed in ischemic-reperfused and cryoinjured cardiomyocytes in order to reduce proliferation of T lymphocytes and protect the myocardium [15]. ICI therapy may reverse these effects, which may partially contribute to ICI-associated myocarditis.

The Dallas criteria, set forth in 1986 as a standard with which histopathologists could diagnose myocarditis, require an inflammatory infiltration and myocytes necrosis or damage not characteristic of an ischemic event [16]. However, the usefulness of the Dallas crietria is limited because of high interobserver disagreement, low sensitivity due to sampling errors, and poor correlation between clinical presentation and prognosis [17,18]. Although endomyocardial biopsy remains the gold standard for the diagnosis of myocarditis, it is necessary to incorporate clinical presentation and imaging findings and to utilize more sophisticated tests, including serological studies and polymerase chain reaction analysis for viral genomes, to identify the disease early.

Conclusion

Despite being a rare irAE, myocarditis is one of the most fatal side effects of ICI treatment. Because of its high mortality rate, it is crucial to recognize myocarditis at an early stage. Patients presenting with neuromuscular symptoms should be closely monitored and evaluated for potential cardiotoxicity. Currently, the histopathological features of ICI-induced myocarditis are not well characterized because of the limited number of cardiac biopsies and autopsies performed. Although lymphocytic myocarditis is invariably observed, other findings, such as giant cell formation and endothelitis, are not consistently identified. This may be owing to sampling error or biopsies collected at different phases of the disease. This emphasizes the importance of 1) obtaining endomyocardial biopsies or autopsies to better discern the specific immune responses that cause myocarditis and of 2) subtyping of T cells and determining PD-L1 expression, which may have important roles in the characterization of myocarditis and responses to therapy. Taking these steps will lead to the development of more targeted therapies for irAEs besides general immunosuppression with steroids. With the accelerating progress in the field of immunotherapy, there is a pressing need to better understand the underlying mechanistic injury process and to develop novel therapeutic approaches to address potentially fatal complications.

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