Diagnosing Immune Checkpoint Inhibitor-Induced Myocarditis: Insights, Challenges, and Uncertainties

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Abstract

Recent advancements in immunotherapy have substantially improved overall survival and quality of life among patients with cancer. Notably, immune checkpoint inhibitors (ICIs) have emerged as a revolutionary strategy, particularly in the management of advanced cancers. However, the success of ICIs is accompanied by the challenge of immune-related adverse events. Although rare, cardiovascular adverse events associated with ICIs are associated with high fatality rates and rapid clinical progression, thereby necessitating timely intervention. This review explores the histopathologic characteristics of ICI-induced myocarditis, shedding light on the complexities of diagnosis and management. Several studies examining the histopathologic features of ICI-induced myocarditis have emphasized the roles of macrophages and the potential utility of ancillary tests such as immunohistochemistry. Quantifying CD68+ macrophage abundance may enhance diagnostic sensitivity, thereby providing valuable insights into clinical outcomes. In conclusion, this review underscores the need for a nuanced approach to diagnosing ICI-induced myocarditis. The comprehensive exploration of histopathologic characteristics, ancillary tests, and emerging diagnostic markers provides valuable guidance for practicing pathologists. As the population of ICI-treated patients with cancer continues to grow, optimizing immunohistochemistry panels and refining diagnostic criteria will be crucial to address the unique challenges posed by ICI-induced myocarditis.

Keywords: Cardiovascular histopathology; onco-cardiology/cardio-oncology; immune checkpoint inhibitor

Introduction

Recent advances in cancer therapy have substantially improved overall survival [1] and quality of life [2] among patients with cancer. By harnessing the immune response against tumor cells, immune checkpoint inhibitors (ICI) have revolutionized cancer management strategies, particularly for advanced cancer. However, although ICIs offer a promising avenue to improve clinical outcomes in cancer patients, many of their adverse effects,
driven by the same immunologic mechanisms that exert cytotoxic effect on tumor cells, may unleash an uncontrolled immune response to the body’s native cells, thus leading to adverse events.

Cardiovascular adverse events, including myocarditis, pericarditis, and vasculitis, are uncommon manifestations of ICI-related toxicity and are estimated to affect fewer than 1% of patients [3]. Although rare, ICI-related cardiovascular adverse events are associated with high fatality rates (reported range 25%–40%) [4] and a rapid clinical course, thus requiring emergent intervention and timely diagnosis. However, the definitions of cardiotoxicity can be highly variable and obscure, and diagnosing myocarditis is clinically challenging in patients with cancer under active treatment. The true incidence and prevalence of cardiac adverse events are likely to be underreported [5].

Endomyocardial biopsy is widely recognized as the gold standard for diagnosing myocarditis (Figure 1) [6]. Despite being considered a relatively safe procedure (complication rate ~1.9% [7]), its invasiveness is inherently associated with a risk of complications. A lack of expertise (among both treating clinicians and interpreting pathologists) at many institutions further renders endomyocardial biopsy an underused diagnostic tool. The interpretation of endomyocardial biopsies is additionally complicated by variability in tissue preparation methods, the availability of ancillary studies (immunohistochemistry, immunofluorescence, and electron microscopy), and differences in guidelines/reporting (Dallas Criteria in North America vs. European Society of Cardiology [ESC] in Europe) [8]. Furthermore, no guidelines have been established for the histopathologic diagnosis of ICI-induced myocarditis. Several recent studies have described the complex and heterogeneous histopathologic findings in ICI-induced myocarditis, and demonstrated the utility of ancillary tests. The aim of this brief review is to summarize the salient features and provide a reference for practicing pathologists to interpret endomyocardial biopsies in the setting of clinically suspected ICI-associated myocarditis.

**Diagnostic Challenge: Heterogeneity in Clinical Presentation**

ICI-associated myocarditis can have a variety of clinical presentations. In mild cases, patients may present with asymptomatic cardiac biomarker elevation (troponin and BNP) [9]. In a recent multicenter Franco-German study, Troponin-T has been shown to be a more sensitive biomarker than Troponin I and creatine kinase in diagnosing ICI-associated myocarditis [10]. The most concerning cases involve severe decompensation with end-organ damage, and common clinical presentations include chest pain, dyspnea, and palpitations [11]. The primary diagnostic challenge in clinical practice is that similar symptoms may be observed in other cardiac (viral myocarditis, coronary artery disease, and pericarditis) and non-cardiac etiologies (pulmonary embolism and pneumonitis) [9]. Although ECG may contribute to diagnosing myocarditis, the findings
are usually not specific [12]. Cardiac magnetic resonance imaging is considered the most effective non-invasive study for diagnosing myocarditis [13], and recent updates to the Lake Louise Criteria have improved this method’s diagnostic accuracy [14]. Coronary angiography is frequently performed with endomyocardial biopsy to rule out coronary artery disease. Cardiac magnetic resonance [15] or endocardial voltage mapping [16] guidance may improve the diagnostic yield of endomyocardial biopsy.

**Lessons from Recent Case Series**

An early systemic study compared the histopathology findings of ICI myocarditis (including nine endomyocardial biopsies and one autopsy) with those of cardiac transplant allograft rejection. Champion and Stone [17] successfully used a CD3+ cell count (>50 cells/HPF) to dichotomize the study population into high- and low-grade groups. Furthermore, ICI myocarditis was found to feature more lymphohistiocytic inflammation, rather than a lymphocytic pattern in patients with acute cellular rejection after cardiac transplantation. Interestingly, low-grade ICI myocarditis cases were associated with elevated PD-L1 positive macrophages. PD-L1 staining in myocytes was 100% sensitive in ICI myocarditis cases. However, its specificity was suboptimal: approximately half the control group (grade 2R acute cellular rejection) also showed positive PD-L1 staining. Nonetheless, use of a higher threshold (>10 cells/10 HPF) enhanced its diagnostic utility in distinguishing high-grade ICI (two of three cases) from low-grade ICI myocarditis or acute cellular rejection. Recently, Jimenez et al. [18] have demonstrated that characterizing the presence of interstitial macrophages, in addition to using the Dallas criteria, can enhance the diagnostic yield of ICI-associated myocarditis; these findings correlate well with clinical parameters. Incorporation of histiocytic inflammation (>50 CD68+ cells/HPF) would have led to reclassification of 7/15 patients into the definitive category. These two studies highlight the role of macrophages in diagnosing ICI-associated myocarditis.

Another multicenter collaboration [19] among six clinical centers in Germany and the United States has further underscored the heterogeneities in the clinical and histopathologic presentation of ICI-related myocarditis. On the basis of clinical criteria, eight patients with melanoma treated with ICI with reported cardiologic adverse events were included in the study. Histopathologic findings were reported on six patients, including four biopsies and two autopsies. Whereas several cases showed non-specific findings, such as interstitial fibrosis or scattered inflammatory cells, cardiomyocyte damage was reported in one biopsy with lymphohistiocytic infiltrates, and one autopsy case exhibited diffuse myocarditis with multinucleated giant cells, lymphocytes, and eosinophils. Although viral testing was not explicitly described in this autopsy case, a more recent study [20] has demonstrated the presence of enterovirus in an endomyocardial biopsy from a patient with ICI-related giant cell myocarditis, with immunohistochemical evidence of viral protein synthesis in cardiomyocytes surrounded by inflammatory infiltrates. A plethora of preclinical and clinical studies have suggested that CTLA-4 inhibition is associated with giant cell myocarditis with predominant CD4+ cell infiltration, whereas PD-1 inhibition is more frequently associated with lymphocytic myocarditis mediated by CD8+ T cell infiltration [21]. These studies have suggested that the complex interactions among the immune system, infections agents, and ICIs in the myocardium remain a challenging area for further investigation.

Palaskas et al. [22] have proposed a grading system modified from the Dallas criteria and World Heart Federation criteria according to additional immunohistochemistry studies. Similarly to the results reported by Champion and Stone, PD-L1 staining was observed in infiltrating macrophages and damaged cardiomyocytes. Although the proposed classification system did not show a significant stratification of overall mortality, this study demonstrated a spectrum in the density of inflammatory cell infiltrates in patients with clinically suspected ICI myocarditis. With an aggressive institutional protocol aimed at early diagnosis of myocarditis in the setting of rising troponin levels, this single-center study captured milder histologic findings than observed in other case series. Four patients with histologic evidence of myocardial inflammation continued on ICI therapy. Those four patients included three patients with grade 1A (10–20 inflammatory cells/HPF without myocyte injury) and one patient with grade 1B (>20 inflammatory...
cells/HPF without myocyte injury); no fatalities were reported. Whether this mild inflammation is a harbinger of evolving myocarditis or merely reflects a self-limited process warrants further investigation. Notably, an imminent decision point would be the cessation of ICI therapy and/or immunomodulatory agents in light of mild inflammation. To address these questions, future studies following a multidisciplinary approach will be necessary to maximize the therapeutic effects of ICIs and circumvent the collateral damage from systemic toxicity [23].

In a smaller cohort, Balanescu et al. [24] have observed extensive lymphohistiocytic inflammation with evidence of myocyte necrosis in two of three patients clinically diagnosed with myocarditis. In areas of cardiomyocyte damage, positive C4d and PD-L1 were present, thus further supporting the utility of these two markers in the assessment of myocyte injury. One of the three patients did not show histopathologic evidence of myocardial inflammation – a finding attributed to tissue heterogeneity and sampling bias.

A rare syndromic presentation seen in ICI-treated patients is ICI-induced myocarditis with myositis and/or myasthenia gravis overlap syndrome (IM3OS). Pathak et al. [25] have systematically reviewed 60 recent cases reported in prior studies, only 15 of which received cardiac biopsies. Whereas most cases showed lymphocytic infiltrates with predominantly CD4+ and CD8+ T-cells and CD68+ macrophages, cardiomyocyte injury was observed in only six cases, thus again underscoring the known limitations of the Dallas criteria [26]. Endocardial fibrosis [27] has been described in a case of advanced thymic malignancy, although echocardiography did not reveal any imaging evidence of fibrosis. Muscle biopsies from 18 cases showed various degrees of myofiber necrosis and atrophy, with predominantly lymphocytes and macrophages. Interestingly, whereas negative staining for CD20 was seen in two case reports [3, 28], a single-center study [29] from Japan has reported CD20+ cell infiltration in two patients with a clinical diagnosis of myositis overlapping with myocarditis.

**Roles of Macrophages**

In the native myocardium, most immune cells are tissue resident macrophages [30, 31], which are key regulators of cardiac inflammation [32]. Increases in macrophages are observed in a variety of physiological and pathologic conditions, including aging, hemodynamic stress [33], acute myocardial infarction, heart failure, and COVID-19 [34]. Cardiac macrophages and recruited monocytes play divergent roles, including both exacerbation and attenuation of tissue injury [35]. According to current clinical diagnostic guidelines [28], tissue diagnosis alone is confirmatory for definite myocarditis [11]; however, the Dallas criteria do not explicitly account for the presence of macrophages as a component of inflammatory infiltrates, and the ESC guidelines [36] specify “up to 4 monocytes/mm² with the presence of CD3 positive T-lymphocytes ≥7 cells/mm².” Future investigations are warranted to explore and establish the roles of macrophages and associated diagnostic markers, including CD68 and CD163 (a marker with a higher specificity for the macrophage/histiocyte/monocyte lineage [37]) in the workup of ICI-associated myocarditis.

**Myocyte Injury**

Myocyte injury is conventionally assessed according to histologic features of myocytolysis. For instance, the International Society for Heart and Lung Transplantation has described clearing of sarcoplasm/nuclei, enlarged nuclei, and conspicuous nucleoli [38], although in daily practice, identification of the presence of myocyte injury can be subjective and controversial [8, 39]. In a recent trans-Atlantic survey [8], approximately half the participating cardiovascular pathologists indicated that they would make a diagnosis of myocarditis in the absence of myocyte injury.

Two main systems are used for the diagnosis of myocarditis. The Dallas criteria [40], widely used by North American pathologists, emphasize qualitative, morphologic features (edema, myocyte damage, and inflammatory infiltrates). The cardiovascular pathology community has advocated for redefining the Dallas criteria to encompass additional modalities including immunohistochemistry, polymerase chain reaction, and cardiac antibody assessment [26]. Assessment of myocyte injury has been shown to be a major area of discrepancy in distinguishing borderline myocarditis from myocarditis or non-myocarditis [39].
In contrast, the ESC guidelines [38] use a more quantitative method with immunohistochemical assessment. Compared with the Dallas Criteria, the ESC guidelines are more conducive to diagnosing ICI-associated myocarditis, although certain revisions might be necessary to account for the lymphohistiocytic cell populations. Immunohistochemical markers for cardiomyocyte injury, such as C4d and C9 [41], and the accumulation of fibronectin and depletion of troponin T [42], might enhance sensitivity in diagnosing myocyte damage and potentially decrease interobserver variability.

Antigen Presentation

Infiltrating immune cells are key mediators in the tumor microenvironment. A case report by Johnson et al. [43] included two patients with melanoma who developed fatal myocarditis after treatment with ICI. Pre- and post-treatment biopsies were compared; both patients’ pre-treatment biopsies showed minimal to modest amounts of immune infiltrates, and postmortem assessment revealed prominent lymphocytic infiltrates. Interestingly, one of the two patients had clonal expansion of T-cells; the clonal T-cell populations sampled from the myocardium were identical to those infiltrating the tumor and skeletal muscle. These findings suggested the possibility of a shared epitope/antigen between the tumor and striated muscle. The anti-tumor response might have caused collateral damage in skeletal muscles and cardiomyocytes. Various strategies have been developed to enhance antigen presentation in cancer immunotherapy [44, 45], and the potential for fine-tuning antigen presentation to minimize off-target effects while designing more tumor-specific immune response merits further investigation. In such endeavors, an organoid-based screening tool [46] and engineered heart tissue [47] might provide a physiologic platform recapitulating the complex interactions among tumors, immune cells, and the myocardium.

Immune Profiling: Bench to Bedside

A pioneering mechanistic study has investigated the immunoproteasome, a key modulator in ICI-related myocarditis, acting through a troponin I-directed autoimmune pathway [48]. Through mitigating cytokine production, immunoproteasome inhibitors have been shown to attenuate the ICI-related heart-specific autoimmune response in mouse models. In two patients with ICI-related myocarditis, bulk RNA-sequencing analyses have demonstrated enrichment of various markers indicating immunoproteasome expression, TLR-mediated activation of monocytes, chemokine and cytokine responses, as well as T- and B-cell activation.

Recent advances in single-cell profiling techniques, including time-of-flight mass cytometry (CyTOF), single-cell RNA sequencing (scRNA-seq), single-cell T-cell receptor sequencing (scTCR-seq), and cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq), offer further options to study tumor cells and their immune microenvironment in detail [49]. By bridging gene expression data and the histologic characteristics of cell groups in a tissue section, spatially resolved transcriptomics and proteomics will provide another powerful tool for deciphering the molecular mechanisms of ICI-induce myocarditis and personalizing onco-immunotherapy.

Conclusion

Although most ICI-associated cardiac adverse events are characterized within the first 90 days of therapy initiation, increasing evidence demonstrates late-onset cardiac adverse events with more pronounced left ventricular systolic dysfunction and heart failure, and less frequent supraventricular arrhythmias [50]. Fulminant myocarditis has been reported as many as 2 years after ICI initiation [51]. Although endomyocardial biopsy is usually performed in patients with high clinical suspicion for ICI-induced myocarditis, other differential diagnoses including immune-related sarcoidosis [52], viral myocarditis [12], and vaccination [53] must be considered.

Optimizing the IHC panel for ICI-induced myocarditis is crucial to address the rising prevalence of ICI-treated patients with cancer in an aging population. Although no consensus exists, many studies [17, 18] have described lymphohistiocytic inflammation as the key finding in ICI-induced myocarditis. Macrophage markers (CD68, CD163, and
PU.1) and lymphocytic markers (CD3 and CD20) may help reveal the inflammatory cell population. Overall, collaborative efforts among cardiovascular pathologists, clinicians, radiologists, and scientists are needed to establish a robust, consensus-driven diagnostic framework to diagnose ICI-induced myocarditis and ultimately improve the clinical outcomes of patients with cancer.

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Conflicts of Interest

The authors have no conflicts of interest to declare.

REFERENCES


