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2016-11-29


http://hdl.handle.net/10138/230026
https://doi.org/10.1016/j.jacc.2016.09.937

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The Quest for New Approaches in Myocarditis and Inflammatory Cardiomyopathy

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ABSTRACT

Myocarditis is a diverse group of heart-specific immune processes classified by clinical and histopathological manifestations. Up to 40% of dilated cardiomyopathy is associated with inflammation or viral infection. Recent experimental studies revealed complex regulatory roles for several microribonucleic acids and T-cell and macrophage subtypes. Although the prevalence of myocarditis remained stable between 1990 and 2013 at about 22 per 100,000 people, overall mortality from cardiomyopathy and myocarditis has decreased since 2005. The diagnostic and prognostic value of cardiac magnetic resonance has increased with new, higher-sensitivity sequences. Positron emission tomography has emerged as a useful tool for diagnosis of cardiac sarcoidosis. The sensitivity of endomyocardial biopsy may be increased, especially in suspected sarcoidosis, by the use of electrogram guidance to target regions of abnormal signal. Investigational treatments on the basis of mechanistic advances are entering clinical trials. Revised management recommendations regarding athletic participation after acute myocarditis have heightened the importance of early diagnosis. (J Am Coll Cardiol 2016;68:2348–64) © 2016 by the American College of Cardiology Foundation.
Histologically verified cardiac sarcoidosis (CS) was diagnosed in 233 patients in Helsinki, Finland (8). The annual detection rate of CS was 0.6 per 100,000 adults (>18 years of age) in the last full 2-year study period between 2013 and 2014. The prevalence of CS in 2012 was 2.2 per 100,000 people. Over the study period, the detection rate of CS increased more than 50-fold. From case series, CS affects 2.3% to 11% of patients with sarcoidosis and 2.5% of unexplained cardiomyopathy in a referral population (diagnosed by heart biopsy, 31 of 1,235). Asymptomatic sarcoid heart involvement is more common. In autopsy studies, myocardial involvement has been seen in 20% to 27% of consecutive sarcoidosis patients (9). Also, other granulomatous systemic diseases may involve the heart in a subclinical way. In antinuclear cytoplasmic antibody-associated vasculitis, cardiac involvement is found in up to 65% of patients in remission, the majority caused by nonischemic injury of the heart (10).

GCM is a rare, rapidly progressive, and clinically important form of myocarditis (11). The estimated annual detection rate for GCM in Finland between 2013 and 2014 was 0.13 per 100,000 people (12,13). Over a 23-year period between 1991 and 2015, 55 cases of GCM were diagnosed. The detection rate increased 10-fold over this period. The cause for the increased rate of detection of CS and GCM in Finland is uncertain, and may be related to increased awareness of the diseases, increased referral to an advanced heart failure center, or an increased use of biventricular biopsies over the study timeframe. In autopsy registries, histological evidence of GCM was found in up to 6.6 per 100,000 people or 3% to 5.6% of all myocarditis autopsies (14,15). In endomyocardial biopsy (EMB) studies of dilated cardiomyopathy (DCM) at a tertiary referral center, GCM was found in 0.2% to 1.2%, and 2% of cardiac transplant patients had GCM (16).

ETIOLOGY

The most commonly identified pathogens in acute myocarditis are viruses (17). The immune response triggered by injury and autoantigen-specific mechanisms leads to organ dysfunction, pathological remodeling, and heart failure. From the 1950s to the 1990s, enteroviruses were the most frequently identified pathogen, particularly coxsackie virus in North America and Western Europe (18,19). In the past 2 decades, polymerase chain reaction and in situ hybridization have identified a range of cardiotropic viruses, including H1N1 strains of influenza (20), adenovirus (21,22), hepatitis C (23,24), cytomegalovirus, echo-virus, parvovirus B-19, and Epstein-Barr virus (25) in heart biopsy samples. In subjects with more chronic symptoms and “inflammatory cardiomyopathy,” parvovirus B19 (26) and human herpes virus 6 genomes (27) predominate.

In select populations, specific nonviral infections and autoimmune syndromes remain important causes of myocarditis. Autoimmunity following untreated streptococcal infection results in rheumatic carditis (28). Bacteria, such as diphtheria and Borrelia burgdorferi (Lyme disease), or parasites, such as

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**FIGURE 1** Myocarditis and Cardiomyopathy Deaths and Death Rates From 1990 to 2015

(A) Number of global deaths with 95% uncertainty interval for women (orange) and men (blue) due to cardiomyopathy and myocarditis from 1990 to 2015. (B) The global death rate per 100,000 people with 95% uncertainty interval for women (orange) and men (blue) due to cardiomyopathy and myocarditis from 1990 to 2015. From the Global Burden of Disease Project, Institute for Health Metrics and Evaluation database. Image provided by Greg A. Roth, MD, MPH, and Catherine O. Johnson, Division of Cardiology, University of Washington, Institute for Health Metrics and Evaluation.
Trypanosoma cruzi (Chagas disease), are important in specific regions (29,30). Hypersensitivity myocarditis may result from drug- or vaccine-related heart-specific autoimmunity. Methylpapa, hydrochlorothiazide, furosemide, ampicillin, tetracycline, azithromycin, aminophylline, phenytoin, benzo diazepines, tricyclic antidepressants, and tumor necrosis factor antagonists all have a low rate of myocarditis (31). Although most cases of hypersensitivity myocarditis develop early in the course of drug use, up to 15% of clozapine-induced myocarditis develops later, up to 2 years after initiation of drug therapy (32,33). Vaccination against smallpox infection is associated with myopericarditis in up to 6 per 10,000 vaccines (34). Other noninfectious causes of myocarditis include cardiac inflammation in the context of radiation or systemic autoimmune diseases, including antineuclear antibody-related vasculitis, systemic sclerosis, lupus erythematosus, and celiac disease (35). A total of 20% of GCM patients have a history of other autoimmune disorders. In GCM, the risk of transplantation and death has decreased since the introduction of prompt calcineurin inhibitor-based immunosuppression (12). Biopsy-documented GCM recurs up to 8 years after successful treatment in 12%, in both the native heart and in the allograft following transplantation (36).

PATHOGENESIS

Most of the current mechanistic understanding of human myocarditis derives from experiments in susceptible rodent strains. Inflammatory cellular infiltrates develop either after cardiomyocyte infection with a cardiovirulent virus strain (37,38), parasites, or bacteria, or after delivery of cardiac antigens with a strong adjuvant or carried within dendritic cells in noninfectious models (39-41). Models that do not rely on infection only provide insights to the latter phases of myocarditis.

The transition from acute viral infection through active inflammation to DCM can be conceptualized as a multiphase model (Central Illustration). The opportunities for therapeutic intervention vary by stage of disease. Depending on the inciting infective agent, the mechanisms of early tissue injury as well as the resulting patterns of inflammatory mediators and cytokines differ. In virus-mediated myocarditis, for example, viral entry into cardiomyocytes leads to the production of type 1 interferons within hours and to myocyte death through apoptosis and autophagy (42). It requires specific cell surface receptors, such as the coxsackie-adenovirus receptor and decay-accelerating factor (CD55) for some coxsackie B virus strains (43,44). The activity of inflammatory signaling pathways in cardiac myocytes contributes to cox- sackie B virus replication via effects on viral replication (45,46). The result of the initial damage is presentation of normally sequestered cardiac protein fragments in the context of tissue damage. The pathogenesis of myocarditis varies by pathogen. For example, in Chagas myocarditis, tissue damage results not only from the associated invasion of inflammatory cells, but also directly, through parasites releasing bioactive compounds and promoting oxidative stress (47). Cardiac parasympathetic neurons are also damaged by the parasite, leading to unopposed sympathetic activation (48).

A second phase that evolves over hours to days involves activation of an innate immune response, consisting of nitric oxide, altered regulatory T-cell function, natural killer (NK) cells, release of mediators such as type I interferon in viral myocarditis (49-51), or granule components from eosinophils and polymorphonuclear cells in bacterial and parasitic myocarditis. The exodus of antigen-bearing cells to regional lymph nodes within the appropriate cytokine milieu triggers a third phase, characterized by cellular cardiac infiltrates with pathogen- and autoantigen-specific T cells, macrophages, and antibodies (52-54). Efficient clearance of the infective agent usually results in recovery of normal cardiac function. In genetically susceptible experimental animals and humans, a breakdown of T-cell tolerance to cardiac self-antigens may occur (37,55,56). This leads to a process of chronic autoantigen-driven inflammation, which can progress to DCM and end-stage heart failure (57-60). The significance of cardiac autoimmunity in disease progression depends on genetic susceptibility of the host, genetic variations of the inciting microbes, molecular mimicry between cardiac structures and microbial proteins, and environmental factors. Phases of autoimmune inflammation progression cannot be triggered only through infective agents, but also through noninfective microbial particles, heat shock proteins, or immunogenic fragments released after tissue injury, drug exposure, or in the context of systemic autoimmune diseases. In the transition from acute inflammation to chronic fibrosis, inflammation can persist in the absence of symptoms (61,62).

CELLULAR AND EXTRACELLULAR COMPARTMENTS OF CARDIAC INFLAMMATION

Similar to other organs, different cellular compartments are involved in myocardial inflammation and remodeling: 1) the bone marrow-derived
compartment, including effector cells of adaptive immunity, such as T- and B-cells, and a myeloid-derived population of astonishing plasticity, including subsets of macrophages, dendritic cells, granulocytes, eosinophils, mast cells, and an immature precursor population with the capacity to differentiate not only into inflammatory cells, but also into myofibroblasts; 2) the endothelial compartment, which serves as a critical barrier regulating the access of circulating bone-marrow-derived cells to the heart; 3) the interstitial compartment, consisting of fibroblasts, myofibroblasts, and other stromal cells, embedded in an interstitial matrix that contributes to and interacts with the specific local milieu to shape the inflammatory phenotype; and, finally, 4) the cardiomyocyte compartment, which is not only a target of early infection, but also shows specific adaptive responses to inflammatory stimuli, such as hypertrophy and altered calcium signaling (63,64). Changes in these cellular compartments following an inciting event of heart tissue damage, incurred by infections, toxins, ischemia, or immunological imbalance, define the pathological anatomic phenotype of the inflamed heart. Changes in the cellular compartments, including activation, deactivation, or transdifferentiation of specific cell subsets, are regulated by cell-cell interactions and by autocrine and paracrine mediators, including chemokines, cytokines, hormones, and degradation or danger products, acting to activate or inhibit gene expression through more or less specific receptors and their intracellular downstream pathways (65).
Other cellular regulatory mechanisms, such as gene silencing, contribute to the post-transcriptional regulation of myocarditis (62). The extracellular matrix, an intricate proteinaceous network that fills the extracellular spaces and provides structural support and tissue organization, is the fifth element that modulates cardiac inflammation, remodeling, and tissue fibrosis (Figure 2) (66,67).

**INNATE MECHANISMS IN MYOCARDITIS AND INFLAMMATORY CARDIOMYOPATHY**

Cells of the innate immune system mainly include macrophages, granulocytes, dendritic cells, histiocytes, mast cells, and eosinophils. These cells present a broad array of intracellular and surface pattern-recognition receptors that recognize conserved microbial structures that are not shared by host molecules (pathogen-associated molecular patterns) (68).

In myeloid cells, these pathogen-associated molecular patterns induce the inflammasome, a multiprotein oligomer including caspase 1, PYCARD, and nucleotide-binding oligomerization domain (NOD)-like receptors. Pathogen-associated molecular patterns also form inflammasomes by binding to intracellular pattern recognition receptors, such as NOD-like receptor subsets, and to caspase-1. The inflammasome activates the caspase-1 cascade, leading to production of proinflammatory cytokines. In contrast, Toll-like receptors (TLRs) represent a family of pattern recognition receptor molecules that are usually expressed on the cell surface (Figure 2).

TLRs play a key role in the early activation of the innate immune response against viral and other infections. TLRs are predominantly, but not exclusively, expressed in macrophages and dendritic cells. Eleven TLRs have been identified in humans. Thus far, specific roles in inflammatory heart disease and myocarditis...
have been attributed to TLR2, TLR3, TLR4, TLR7, and TLR9, and their downstream adaptors, MyD88 and TRIF (45,69-73). Accordingly, TLR3-deficient mice show markedly increased mortality after infection with enteroviruses (74). Interestingly, a common TLR3 polymorphism has been found in patients with enteroviral myocarditis cardiomyopathy (75). Expression of the TLR3 variant results in significant reduced TLR3-mediated signaling after exposure to enterovirus-specific pathogen-associated molecular patterns, and promotes viral replication. In non-ischemic cardiomyopathy, cardiac expression of pro-patterns, and promotes viral replication. In non-

Expression of the TLR3 variant results in significant reduced TLR3-mediated signaling after exposure to enterovirus-specific pathogen-associated molecular patterns, and promotes viral replication. In non-

Activation of TLRs and the inflammasome results in release of cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-18, or high-mobility group box 1 protein in myocarditis and DCM (77,78). TLR2 and TLR4 activation mediates TNF release, and TLR2, together with TLR4, promotes IL-1β secretion. Moreover, self-proteins, such as cardiac myosin, can directly activate TLR2 and TLR4 (79). TLR4 is up-regulated in macrophages and mast cells during the early innate immune response following acute coxackie virus B3 infection. In patients with myocarditis, TLR4 messenger ribonucleic acid (mRNA) expression is up-regulated and directly correlates with viral ribonucleic acid (RNA) levels. However, TLR4-deficient mice show reduced cardiac inflammation and IL-1β levels in the heart during acute coxackie virus B3 myocarditis. The IL-1β axis is directly involved in cardiac fibrosis, a hallmark of pathological remodeling and heart failure progression (61). In contrast, high-mobility group box 1 protein promotes cardiac inflammation in a mouse model of troponin I-induced experimental autoimmune myocarditis by a complex interaction involving the receptor for advanced glycation end products and TLRs (77).

**ROLE OF MACROPHAGE SUBSETS IN MYOCARDITIS DEVELOPMENT**

Mononuclear phagocytic cells, termed macrophages, play an important role as scavengers, microbicidal effectors, and regulatory cells in cardiac inflammation. Cells of the monocyte and macrophage lineages represent the majority of infiltrating inflammatory cells in human and experimental myocarditis. The spleen is an important reservoir of monocyte precursors in inflammatory conditions. Cardiac injury upon myocarditis results in early recruitment of Ly6Chigh inflammatory macrophages (80,81). Blockade of chemokines associated with the recruitment of Ly6Chigh inflammatory monocytes, such as the CCR2 ligands CCL2/MCP1 or CCL3/MIP1α, ameliorates autoimmune myocarditis (82,83).

CD4- T (type 1 T helper [Th1]) cells strongly influence the functional differentiation of monocytes toward proinflammatory or classical M1 macrophages (84). The Th1 IFN-γ potentiates the microbicidal activity of macrophages and, as such, promotes antigen presentation (85).

Alternatively-activated Ly6Chigh M2 macrophages predominate during the process of myocardial healing (86,87). These M2 macrophages blunt the inflammatory response and promote cardiac fibrotic healing (68,86). Here, Th2-associated cytokines, mainly IL-4 and -13, activate the anti-inflammatory M2 macrophage phenotype (87). These M2 cells are also hallmarks of the transition from acute myocarditis to chronic pathological remodeling, where the macrophages are replaced by profibrotic myofibroblasts (88,89). Overall, macrophage phenotypes in vivo are variable, due to their high plasticity and capacity to adopt varying degrees of specialization, including microbicidal activity, antigen presentation, immunoregulation, and fibrosis, depending on the local cytokine and chemokine milieu.

**ROLE OF T-CELL SUBSETS IN MYOCARDITIS**

T cells play a central role in the development of myocarditis, despite the fact that they account for a minority of heart infiltrating cells in mouse models of myocarditis, as well as biopsy-proven human myocarditis (90). CD4+, as well as γδ T cells, CD8+ T-cell, and NK cell responses are critical to overcome early acute viral infections in rodents and humans (53,91,92). Importantly, T-cell responses, in particular CD4+ cells, can turn initial myocarditis into an autoimmune response, even after clearance of the infectious agent (56), in part due to thymic intolerance to alpha-myosin heavy chain (α-MyHC) (93). Activated, self-antigen-expressing dendritic cells promote expansion of autoreactive CD4+ T-cells and heart-specific inflammation (39,94). Transgenic mice overexpressing α-MyHC-specific T-cell receptors (TCR) in nearly all CD4+ T cells develop spontaneous autoimmune myocarditis, even in the presence of minimal antigen-presenting cell activation (95).

CD4+ effector T cells can be divided into 3 major subsets, according to the cytokines they produce: Th1 CD4+ cells mainly produce IFN-γ; Th2 CD4+ T cells mainly produce IL-4, -5, and -13; and Th17 CD4+ T cells mainly produce IL-17. Those subsets play distinct roles in myocarditis and its progression to
DCM (45,96–98). IFN-γ-producing CD4+ T cells predominate in autoimmune myocarditis in mice and human patients, the latter in α-MyHC-responding CD4+ T cells. Also, IFN-γ-overexpressing mice develop spontaneous cardiac inflammation (95). These findings all point toward a crucial role of IFN-γ-producing T1 CD4+ cells in myocarditis.

Besides promoting inflammation, IFN-γ has a paradoxical role in preventing exaggerated inflammation, as mice undergoing viral, parasitic, and autoimmune myocarditis in its absence present with increased cardiac inflammation (99–101). Mechanistically, IFN-γ is a key mediator of a negative feedback loop limiting activated T-cell expansion. IFN-γ promotes nitric oxide production by TipDCs, a distinct, monocyte-derived CD68+CD11b hiCD11c+ dendritic cell subset (102). Nitric oxide directly induces apoptosis of activated Th1 and Th17 effector helper cell subsets.

Th17 cells have been recognized as an important effector T-cell population in myocarditis. IL-17 depletion results in decreased severity of autoimmune myocarditis in mice (54,103). However, its complete absence in IL-17A knockout mice does not affect myocarditis (104), but is associated with a significant reduction in cardiac fibrosis and progression to DCM (104). Also, mice lacking IL-23 or signal transducer and activator of transcription 3, critical for Th17 differentiation, have reduced severity of myocarditis (97,100). Taken together, Th17 cells and associated cytokines appear as major drivers mainly in chronic myocarditis. Importantly, the cardiac myosin-Th17 response promotes heart failure in human myocarditis (105). In contrast, Th1 cells play a dual role: as mediators in early autoimmune T effector cell expansion, and as regulators through a negative feedback mechanism that protects from overwhelming cardiac inflammation.

Finally, heart-specific, T-helper, cell-dependent immunoglobulin G autoantibodies are present in human and rodent myocarditis and in DCM. Their presence predicts the outcome of DCM patients (106–109). Anti-beta-1 adrenoceptor antibodies are detected in up to 38% of DCM patients (110) and predict the development of cardiac dysfunction in relatives of DCM patients (107). To better understand the role of autoantibodies in myocarditis, comparisons of patient outcomes with and without myocarditis will be needed.

**SEX DIFFERENCES IN MYOCARDITIS AND INFLAMMATORY CARDIOMYOPATHY**

Men are diagnosed with myocarditis more often and have a worse prognosis than women, perhaps due to a more pronounced fibrotic response (111). A key question is whether this difference can lead to sex-specific therapies for acute and chronic myocarditis. In murine models, male subjects have increased coxsackievirus B3-induced myocarditis compared with females, due to differences in innate immune responses to coxsackievirus B3, rather than increased viral replication (112,113). Male mice have increased γδ T cells; increased TLR4+CD11b+ inflammation, including macrophages, neutrophils, mast cells, and DCs; and an increased Th1 response compared with females (114). In contrast, protective Th2 responses, increased B cells, more inhibitory Tim-3+CD4+ T cells, and more T regulatory cells dominate the picture in female animals (114,115). Consistent with these findings, intracardiac macrophages from coxsackievirus B3-infected male mice preferentially expressed inducible nitric oxide synthase, IL-12, TNFα, and CD16/32, markers associated with M1 activation (116,117). In contrast, heart-infiltrating macrophages in female animals showed a M2 activation pattern, including arginase 1, IL-10, and the macrophage mannose receptor expression. Specific targets in the inflamasome are under investigation.

**POST-TRANSLATIONAL REGULATION OF INFLAMMATION IN MYOCARDITIS**

Epigenetic factors influence the expression of polygenic susceptibility. For example, microribonucleic acids (miRNAs) have emerged as epigenetic regulators of the cardiac immune response (Figure 3). miRNAs are noncoding, ~21-nucleotide-long, endogenous, small RNA molecules that post-transcriptionally regulate gene expression by imperfectly binding to the 3′ untranslated region, the 5′ untranslated region, or the coding region within a gene. A single miRNA can modulate the expression of transcripts for different active proteins, which are frequently clustered within a single biological process or pathway, such as inflammation or fibrosis (118,119). They may also result in new therapies, as miRNAs can easily be inhibited by their antisense complements (anti-miRs) injected systemically or locally. For example, the first phase II clinical trial with anti-miRs against miRNA-122 successfully reduced the RNA load of hepatitis C virus in patients with chronic hepatitis C virus infection (120).

Recent translational human and experimental miRNA expression studies support causal links between miRNA dysregulation and human myocarditis, and suggest novel miRNA therapeutic targets. miRNA-155, -146b, and -21 are up-regulated in hearts during acute human and murine coxsackie B3
myocarditis (121). In human right ventricular (RV) myocarditis samples, a total of 107 miRNAs are differentially expressed between viral myocarditis and control hearts. Of these miRNAs, 21 are significantly up-regulated and 37 are down-regulated by more than 1.5-fold. Inhibition of miRNA-155 (121–123), miRNA-21, and miRNA-146b (124) by systemically delivered anti-miRs attenuates cardiac inflammation and myocardial damage in CVB3 or autoimmune myocarditis in mice. Cardiac overexpression of miR-590-3p reduced cardiac injury and dysfunction: it inhibits cardiac nuclear factor-κB p50 expression, suppresses nuclear factor-κB activity, and blocks IL-6/TNF-α expression. Furthermore, in a murine model of *Trypanosoma cruzi*-induced myocarditis, expression of 113 of 641 miRNAs was significantly altered. miR-146b, miR-21, miR-142-3p, miR-142-5p, miR-145-5p, and miR-149-5p correlated with the severity of the disease (125), and are potential therapeutic targets for immunomodulation.

The miRNAs may also modulate the virulence of cardiotropic viruses. Following cardiac infection, the enterovirus CVB3 enters the cardiomyocytes via the coxsackie and adenovirus receptor, replicates, and activates immune pathways. The miRNA-221/222 cluster in cardiomyocytes regulates both virulence and inflammatory pathways in the heart (126). Systemic inhibition of miR-221/222 in mice increases cardiac viral load, prolongs the viremic state, and aggravates cardiac inflammation and injury. miR-221/222 works through targeting proteins that orchestrate viral replication and inflammation, including ETSt/2, interferon regulatory factor 2, Bcl2-like 11, TOX, Bcl2 modifying factor, and CXCL12. Similarly, miRNA-155 inhibits PU.1 and suppressor of cytokine signaling 1 in the heart and, as such, de-represses the production of proinflammatory cytokines, enhancing T-cell and monocyte activation (126,127). Together, these results support the concept that a single miRNA or cluster may orchestrate immune activation and modulate myocarditis.

The miRNAs are under investigation as tools for myocarditis diagnosis and prognosis. In blood, miRNAs reflecting cardiomyocyte injury (including miRNA-208 and -499) are increased in acute myocarditis (128). These cardiomyocyte injury markers are not specific for myocarditis, but are also increased in acute ischemic or hypertensive
cardiac events (128,129). Interestingly, inflammatory miRNAs, including miRNA-155, -146b, and -21, did not increase in blood in acute myocarditis (128), possibly reflecting the lack of cellular release of those miRNAs upon inflammation. In a recent study comparing cardiac miRNA profiles in myocarditis patients with CVB3 persistence to CVB3 clearance, 8 miRNAs (miR-135b, -155, -190, -422a, -489, -590, -601, and -1290) were strongly induced only in the hearts of patients with late viral persistence and progressive cardiac dysfunction (130). Profiling of miRNA groups together with their messenger RNA/protein targets in cardiac biopsies is an area of active investigation.

**CLINICAL PRESENTATIONS AND PROGRESS IN CARDIAC IMAGING**

Acute myocarditis most commonly presents with chest pain or dyspnea. A viral syndrome, such as an upper respiratory tract or gastrointestinal illness, often precedes the clinical syndrome by several days to weeks. Chest pain syndromes may be broadly divided into those resembling pericarditis, with an associated rise in troponin, and those resembling an acute myocardial infarction. Angina may be due to coronary vasospasm or microvascular dysfunction. Myopericarditis recurs in about 11% of patients (131). Palpitations and syncope may result from coronary vasospasm or microvascular dysfunction. Myopericarditis recurs in about 11% of patients (131). Palpitations and syncope may result from coronary vasospasm or microvascular dysfunction.

When myocarditis is suspected, we recommend use of the diagnostic algorithm illustrated in Figure 4. EMB should be performed if the patient requires inotropic or mechanical circulatory support, develops Mobitz type 2 second-degree or higher heart block, has sustained or symptomatic ventricular tachycardia, or fails to respond to guideline-based medical management within 1 to 2 weeks. Disorders that have a greater likelihood of high-grade heart block or ventricular tachycardia include GCM, CS, and necrotizing eosinophilic myocarditis. Other clinical scenarios where EMB may be useful include suspected eosinophilic myocarditis and myocarditis associated with systemic inflammatory disorders. If EMB is infeasible or is not clearly indicated, noninvasive diagnostic criteria may be used to diagnose probable acute myocarditis. A diagnosis of probable myocarditis leads to a recommendation to avoid athletics for at least 3 to 6 months (6) and counseling on the likelihood of left ventricular (LV) and clinical recovery, discussed later (132). At medical centers with specialized expertise in myocarditis care, a tailored evaluation involving specialized studies on biopsy tissue or advanced imaging techniques may be appropriate.

In myocarditis presenting as acute or fulminant DCM with heart failure, cardiac troponins are often detectable in blood and can be supportive of the diagnosis (3). With subacute or chronic myocarditis presentations, biomarkers of myocardial injury are usually normal (17,133). We recommend that cardiac troponin levels be obtained in patients with clinically suspected acute myocarditis. Although most electrocardiographic and echocardiographic changes are nonspecific, speckle-tracking-derived strain may identify early myocarditis (134–136). An echocardiogram should be performed in all patients with clinically suspected myocarditis to exclude hemodynamic, pericardial, and congenital causes of cardiomyopathy.

Newer sensitive and specific cardiac magnetic resonance (CMR) sequences have improved diagnostic and prognostic value. T2-weighted CMR sequences detect edema or water, and T1-weighted sequences detect inflammation or fibrosis. Standard tissue characterization by CMR relies on qualitative visual estimates of signal intensity in native T2-weighted and post-contrast T1-weighted sequences. In contrast, native mapping sequences using T1r, or T2r-weighted techniques allow for quantitative, reproducible, pixel-level relaxation times that are compared with standard values derived from normal subjects. In the largest T1r + T2r mapping trial to date, an elevated native T1 yielded the best diagnostic performance of all CMR parameters in patients with acute symptoms. In patients with <2 weeks of symptoms, native T1 yielded the best area under the receiver-operating characteristic curve (0.82) followed by T2 (0.81). The standard Lake Louise Criteria did not perform as well (0.56). Native T1 had little value; however, in the chronic (≥2 weeks) patient group, these parameters could not differentiate patients with inflammation from those without (137).

The most typical late gadolinium enhancement (LGE) pattern in CS consists of multiple, patchy midmyocardial lesions in a noncoronary (i.e., sparing the endocardium) distribution with septal and RV involvement. Diagnostic findings may fade over weeks to months (138).

Although LGE imaging can help in distinguishing nonischemic patterns of myocyte damage and fibrosis from ischemic injury, T2-weighted and early gadolinium enhancement imaging detect other inflammatory features of edema, capillary leakage, and hyperemia. Myocardial edema in a water-sensitive T2-weighted sequence is a typical feature of active inflammation. Hyperemia is a regular feature of
inflammation that may be imaged noninvasively with early, regional post-gadolinium enhancement (139).

In acute DCM, CMR features suggestive of myocarditis predict a greater likelihood of recovery, defined as a left ventricular ejection fraction (LVEF) >55% after 24 months (138). In a second study of 37 patients with acute myocarditis, patients with greater regional or global $T_2$-weighted “edema-sensitive” sequences also had a significant improvement in LVEF at 12-month follow-up (140). In contrast, delayed gadolinium enhancement has been associated with a higher (3.7%/year) risk of a composite of cardiovascular adverse events. The extent of delayed gadolinium enhancement also predicted a composite endpoint of cardiac death, heart failure hospitalization, ventricular tachycardia, and sudden death (141).

The extent of myocardial LGE is also inversely related to survival free of transplantation and life-threatening ventricular arrhythmias in CS. In 59 patients with CS (38 women, mean age 46 ± 10 years), the extent of myocardial LGE, measured as a percentage of LV mass; the LV and RV volumes and ejection fractions; and the thickness of the basal interventricular septum and the ejection fraction of the RV (p < 0.05 for all). In multivariate analysis, LGE extent remained the only independent CMR predictor of the composite outcome (hazard ratio: 2.22 per tertile; 95% confidence interval: 1.07 to 4.59). The extent of LGE >22% (third tertile) had positive and negative predictive values for the composite endpoints of 75% and 76%, respectively (142).

$^{18}$F-fluorodeoxyglucose (FDG)-positron emission tomography (PET) is also useful for detecting active myocardial inflammation, in particular for suspected CS (143,144). Combining FDG-PET imaging with myocardial perfusion detects active inflammation and scarring. Early in CS, $^{18}$F-FDG-PET may show a focal FDG increase in the absence of a perfusion defect. In a more advanced stage, FDG accumulation may correlate with a perfusion defect. After granulomas have “burned out” or inflammation has been therapeutically suppressed, a perfusion defect may persist without active inflammation. $^{18}$F-FDG-PET imaging may also detect extracardiac inflammation and identify lymph nodes that are amenable to biopsy (145,146).

In addition to diagnostic use, $^{18}$F-FDG-PET can inform prognosis. Mismatch of FDG and perfusion measurements predicts adverse cardiac events, and RV involvement is associated with high risk for
arrhythmias (147). Patients with either a PET mismatch or RV involvement had a 3-fold increase in the rate of adverse events. Newer imaging data from CMR and PET should be integrated with established prognostic risk variables, such as QRS width >120 ms, LVEF, and New York Heart Association functional class (147,148).

**THE ROLE OF EMB**

Although EMB is essential for the diagnosis of specific histological disorders, the broad use of EMB as a gold standard has been limited because of cost, availability of experienced centers, and sensitivity (3,149). The sensitivity of EMB for GCM is approximately 80% to 93% (with repeated LV and RV samples), but is much lower, estimated to be around 20% to 30%, for more common lymphocytic and sarcoid myocarditis due to sampling limitations and focal distribution of sarcoid granuloma and clusters of inflammatory cells (12,150). Immunohistochemistry to visualize specific inflammatory cell types and viral genome analysis have improved the sensitivity to 40% for DCM. An ESC position statement recommends “immunohistochemical criteria,” such as the presence of an inflammatory infiltrate consisting of ≥14 leukocytes/mm², including up to 4 monocytes/mm², with the presence of CD3⁺ T-lymphocytes ≥7 cells/mm² (3). Immunohistochemical criteria seem to be particularly useful in subacute or chronic myocarditis, also called inflammatory cardiomyopathies, where the rate of normalization of LV function is only about 20%. LV biopsy increased the yield in disorders that affect the LV, with a stroke risk of about 1:300 to 1:500 (151). Intracardiac electrograms can increase the diagnostic yield of suspected CS from between 20% and 32% (152,153) to between 60% and 80% (144,154).

Cardiac biopsies may be used to identify and quantify viral genomes. Because serological tests for antiviral antibodies correlate poorly with viral genomes detected on heart biopsies, serology cannot be used to substitute for a biopsy-based diagnosis of viral infection (155). Because viral genomes may be detected in normal hearts, and in ischemic and valvular heart disease (156-159), assays for replicative RNA intermediates (mRNA for deoxyribonucleic acid [DNA] viruses) may be required to define active infection. For example, CpG dinucleotide methylation regulates parvovirus 19 (PVB19) viral genome expression (160). A minimum of 4 samples is needed to achieve an acceptable sensitivity for molecular diagnosis of erythrovirus PVB19 (161,162).

Significant cardiac virus presence (possibly related to the development of cardiac dysfunction) has only been described for PVB19 (>250 copies/µg DNA) and for human herpesvirus 6 (>500 copies/µg DNA). Yet, the simple presence of virus DNA/RNA does not reflect active replication of a virus. Looking for mRNA intermediates to score active virus replication is still not part of routine practice. Only a few studies focused on determining the replicative status of cardiotropic viruses, mainly for the detection of PVB19 mRNA intermediates (162). Active PVB19 replication was only seen in cardiac samples with increased inflammation, either in acute myocarditis or chronic inflammatory cardiomyopathy, and was absent in DCM hearts without inflammation. As antiviral therapies have the highest benefit in patients with active virus replication, its detection would be of great value, but methods are not yet established, standardized, or implemented in clinical practice. In the setting of myocarditis, the presence of multiple viral genomes has been associated with progression to a DCM. Clinical studies of the use of molecular assays for active viral replication to enhance the clinical benefit of EMB are underway. Other features on EMB may affect diagnosis and prognosis. GCM may be distinguished from CS by transcriptome analysis (163). The severity of necrosis and fibrosis are associated with an increased risk of death and transplant in GCM (164).

**HOW TO TREAT MYOCARDITIS?**

For all patients with myocarditis presenting as heart failure with reduced systolic function, we recommend following the current guidelines and scientific statements for heart failure management. The current guidelines emphasize gradual titration of neurohormonal-blocking medications, including inhibitors of angiotensin-converting enzyme, angiotensin-receptor blocking agents, and beta-blockers, to doses used in clinical trials (165,166). Tests for specific causes of myocarditis, such as human immunodeficiency virus, Lyme, or Chagas disease are indicated in patients who have heightened pre-test probability. Serological tests for specific systemic rheumatological conditions, such as lupus erythematosus, may be useful in select groups. The 2015 American Heart Association/American College of Cardiology Foundation scientific statement on sports participation after myocarditis recommends 3 to 6 months abstinence from competitive sports. Before clearance, freedom from exercise-induced arrhythmias and normalization of heart function should be assessed with a symptom-limited exercise test, Holter monitor,
and echocardiogram (6). The treatment of patients with myocarditis depends on the clinical scenario and: 1) the presence or absence of inflammation; 2) the presence or absence of viral infection; and 3) one of the specific histological entities, including GCM, listed in the following text.

**VIRUS-NEGATIVE INFLAMMATION-POSITIVE PATIENTS.** In virus-negative patients with chronic idiopathic DCM and increased cardiac inflammation despite guideline-directed medical treatment, immunosuppression with azathioprine and prednisone for up to 12 months can be an option to improve cardiac function (167,168). To confirm the positive outcomes in the first randomized single-center studies using immunosuppression for virus-negative and inflammation-positive DCM (167,168), a multicenter trial using azathioprine and prednisone for 6 months is underway (NCT01877746). Enrollment criteria include more than 6 months of symptoms and a DCM at the time of screening.

**VIRUS-POSITIVE PATIENTS.** Viral genome analysis of EMBs suggests that up to 70% of chronic DCM patients may carry 1 or more cardiotropic viruses (10). A study of IFN-β in virus persistence-related cardiomyopathy, revealed that IFN-β effectively eliminates enteroviral RNA, with a beneficial effect on New York Heart Association functional class \((p = 0.013 \text{ at follow-up week 12})\), improvement in quality of life (Minnesota Heart Failure score; \(p = 0.032 \text{ at follow-up week 24}\)), and patient global assessment (follow-up weeks 12 to 24; \(p = 0.039\)) (169). However, one-half of patients spontaneously eliminated their enterovirus without specific treatment, suggesting that in addition to the presence of viral genomes, evidence of active virus replication should be a criterion for antiviral therapy. Retrospective case series indicate that patients with chronic cardiomyopathy, a higher virus genome copy number, and evidence of virus replication may benefit from intravenous immunoglobulin (IVIG) (170,171). Although IVIG was ineffective for the treatment of acute DCM in general (172), a single-center randomized trial using IVIG for chronic PVB19-related cardiomyopathy to reduce PVB19 viral load is ongoing (NCT00892112). Chronic viral infection in the heart can cause chest pain or heart failure in the absence of cellular inflammation. The clinical term for a cardiac syndrome associated with being virus genome-positive and without inflammation on biopsy is viral heart disease (17).

Corticosteroid therapy is the mainstay of immunosuppressive therapy in CS, even though evidence is on the basis of retrospective observations. Published clinical series suggest that corticosteroid therapy may halt the progression of LV dysfunction, but there are no data on the effect of immunosuppression on survival (173,174). If treatment is initiated early, development of systolic heart failure seems rare. Observational, uncontrolled data suggest an improvement in LV function with immunosuppression in CS patients with severely impaired LVEF (<35%) (8). Atrioventricular conduction block appears to recover in only 10% to 30% of patients after initiation of steroid therapy.

The treatment of GCM includes immunosuppression. Since the first report of the Multicenter GCM Study Group, treatment has relied on the combination of cyclosporine and prednisone, sometimes with azathioprine (175). Retrospective observations from the Multicenter GCM Registry and a prospective study with repeat biopsies suggest that cyclosporine-based combined immunosuppression attenuates myocardial inflammation and improves clinical outcomes (11). Abrupt cessation of immunosuppression within the first 2 years of treatment has been associated with fatal disease relapse (36).

High-grade heart block in acute lymphocytic myocarditis may be temporary and may require only short-term, transvenous pacing until the acute inflammation subsides. In the setting of likely reversible LV dysfunction associated with acute myocarditis, a wearable external defibrillator vest may be used pending reassessment of LV function. In contrast, high-grade heart block often is permanent, and is associated with frequent ventricular arrhythmias in CS. The Heart Rhythm Society expert consensus statement gave a Class IIa recommendation for an implantable cardioverter-defibrillator in patients with suspected CS, regardless of LV function or reversibility of heart block (145).

**CONCLUSIONS**

In summary, myocarditis represents many diseases with distinct immunophenotypes. Most advances in our understanding of inflammatory heart disease pathogenesis have not yet translated into therapeutic approaches or even better diagnostic tests. Multicenter trials examining pathway- and pathogen-specific treatments are underway presently, using immunohistochemical and molecular analysis as part of the enrollment criteria. Peripheral T-cell, DC, monocyte, and NK-cell activation patterns may differ in myocarditis compared with noninflammatory DCM, or between different viruses and purely autoimmune processes. Multiple groups are searching for
peripheral blood immune markers in proteins, and in coding or noncoding RNAs for diagnosis and to guide emerging therapies, such as RNA interference. The path toward personalized treatment in acute myocarditis requires deep clinical and immunological phenotyping. More specific therapeutic approaches proposed or under clinical investigation include individualized antiviral strategies, miRNA-based gene-silencing strategies, cytokine targeting, and pharmacological modulation to alter tissue monocyte precursors of cardiac fibrosis.

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KEY WORDS heart failure, inflamasomes, macrophages, microRNAs, sarcoidosis, t-lymphocyte subsets, viral infection