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REVIEW AND PERSPECTIVES

The spectrum of myocarditis: from pathology to the clinics

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Abstract

Myocarditis is an inflammatory disease of the myocardium, which may occur in isolation or as part of systemic infectious/immune/autoimmune conditions, characterized by vast aetiologic, clinical and histopathologic heterogeneity. The broad spectrum of myocarditis can be categorized according to the prevalent histopathologic pattern including lymphocytic, lymphohistiocytic, eosinophilic and neutrophilic forms, giant cell myocarditis and myocarditis with granulomata. Diverse histopathologic substrates generally reflect different aetiologies and pathogenetic mechanisms and may be critical to clinical decision-making. Active vasculitis, when present, completes the inflammatory spectrum. Unfortunately, the correlation of histopathologic patterns, clinical presentation and disease course in myocarditis is still largely unresolved, due to limited availability of bioptic samples at specific stages of disease and impracticality of serial sampling. We here review the elements supporting an aetiology-driven diagnostic work-up in myocarditis, emphasizing the importance of integrating pathologic studies with clinical features and information derived from multimodality imaging. Furthermore, we explore myocardial inflammation in genetic cardiomyopathies, its role in driving clinical variability and the potential of transcriptomic and proteomic analysis in our understanding of these complex interrelations.

Keywords Myocarditis · Histopathology · Viruses · Immune-autoimmune · Aetiology-driven diagnostic work-up

Overview

Myocarditis is an inflammatory disease of the heart muscle, which may occur in isolation or as part of multiorgan/systemic immune-mediated disorders or reactions to exogenous/endogenous substances. The exact incidence of myocarditis is unknown, due to a number of variables: heterogeneous clinical presentation mimicking other conditions, lack of consensus on a diagnostic gold standard, varying availability and interpretation of endomyocardial biopsy (EMB) among centres and epidemiologic fluctuations according to geographic region, ethnicity, gender and age. In the last decades, however, the diffusion and technical evolution of cardiac magnetic resonance imaging (cMRI), allowing timely detection of myocardial inflammation, have significantly improved our ability to diagnose myocarditis and led to increased recognition of the disease in the general population. The determinants of clinical presentation and severity of myocarditis are not completely understood but include aetiology, type of myocardial infiltrate, infectious or toxic agent-host interaction, coexistence of other cardiac and systemic disorders and possibly genetic predisposition. Regarding the latter, genetic defects in structural proteins can create a vulnerable myocardium which may increase susceptibility to virus-induced or autoimmune myocarditis [1–3].

The clinical presentation of myocarditis is extremely heterogeneous ranging from chest pain with acute coronary syndrome-like presentation, acute heart failure including cardiogenic shock, chronic heart failure, conduction disturbances and bradyarrhythmias, supraventricular and ventricular tachyarrhythmias and sudden cardiac death. Ancillary symptoms may include fever, loss of appetite and abdominal pain [4]. Cardiogenic shock, requiring pharmacological and/or mechanical hemodynamic support, is the presenting symptom

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in fulminant myocarditis, a specific subtype of acute myocarditis with specific treatment indications and prognostic features [5, 6]. Similarly, sudden cardiac death (SCD) may be the dramatic presentation of acute myocarditis, particularly in young, active patients. In athletes, myocarditis has been occasionally associated with illicit substances abuse. Myocarditis is a frequent cause of myocardial infarction with normal coronary arteries (MINOCA). Although the outcome is favourable in half of the patients, about 25% develop severe haemodynamic or electric instability and an important minority will suffer SCD, fulminant/acute heart failure (in 12–15%) or progressive dilated cardiomyopathy (DCM) eventually requiring heart transplantation [4, 7].

As a consequence of the clinical heterogeneity and complexity of myocarditis, there is still no consensus as to which diagnostic algorithm should be preferred in order to guide management. Numerous areas remain highly controversial in clinical practice, including the determinants of the natural course of myocarditis, the most effective and accurate tools for prognostic stratification and the most appropriate therapeutic strategies in different settings [8, 9]. In most scenarios, both EMB and cMRI are deemed essential to a contemporary diagnosis of myocarditis. While fierce debate continues regarding the pre-eminence of one technique over the other in different clinical scenarios, only the pathologist can provide undisputable diagnoses by histopathological adjudication and molecular analyses. Exploiting decades of pathological exploration, this review aims to provide an insight into the complex world of myocarditis, addressing crucial issues in the current clinico-pathological diagnostic debate with a view to potential contributions from molecular tools such as transcriptomic and proteomic analysis.

In heart transplantation rejection, the immune-mediated myocarditis par excellence, advanced histological and immunohistochemical EMB protocols contributed greatly to excellent therapy results. This model should be remembered when developing further knowledge and diagnostic protocols in myocarditis.

Aetiology and pathogenesis

The causes of myocarditis are extremely varied and include infectious and noninfectious agents [10], as outlined in Tables 1 and 2.

Infectious myocarditis

Infectious aetiologies are the most frequent causes of acute myocarditis/inflammatory cardiomyopathy [8, 9] and include an impressive array of viruses, bacteria, protozoa, fungi and other rare pathogens (Table 1). In North America and Europe, where an aetiological diagnosis is more frequently obtained, the most common causes are viruses [4, 11, 12]; in Africa, Asia and South America, aetiologic data are still scarce. Chagas disease, an endemic parasitic infection caused by *Trypanosoma cruzi*, is the most important infectious myocarditis/cardiomyopathy in South and Central America [13–15], and represents the most prevalent cause of cardiac failure and heart transplantation in Brazil [16].

In recent years, improvements in molecular techniques, including polymerase chain reaction (PCR), and their emerging use in heart tissue, have fostered our understanding of viral myocarditis and its causes. Besides the classic Enteroviruses (most frequently Coxsackieviruses of group B) other viral genomes are found in myocarditis samples, including Parvovirus B19, Epstein-Barr virus, Human Herpesvirus 6, Cytomegalovirus, Herpes Simplex viruses, Influenza viruses, Hepatitis C Virus and Human Immunodeficiency Virus (HIV) [8, 17–22]. Some aetiologies show geographical variations, such as Parvovirus B19 and Herpesvirus type 6, whose genomes have frequently been found in Germany [23–26]. However, there is currently much debate regarding the significance of viral genomes in myocardial tissue and their ability to cause direct infection of myocytes and myocardial damage, as opposed to being innocent bystanders [9, 27–30]. Furthermore, about 30% of patients seem to have multiple infections by various cardiotropic viruses, whose clinical relevance remains ill understood [23].

The majority of information on the pathogenesis of viral myocarditis is supplied by murine enteroviral models, which focused on the significant role of the direct effect of viral infection on cardiomyocytes [31–33]. Although pathogenetic mechanisms may vary according to different virus biologies, generally speaking, the pathophysiology of myocarditis can be divided into three phases [8, 32, 34, 35]. Initially, the virus enters the cardiomyocytes and proliferates causing direct myocyte damage with exposure of intracellular antigens. Following myocyte lysis, the innate immune response and cell-mediated (T and B lymphocytes) immune response is initiated which may contribute to (ongoing) inflammation, also as a consequence of persistent virus infection [32]. At this time, pathogen-specific antibodies cross-reacting with endogenous cardiac epitopes may also appear. In the third stage, removal of the pathogen leads to downregulation of the inflammatory cascade, resulting in subacute/chronic inflammation with additional myocyte necrosis, fibrosis and chamber remodelling. In some patients, the inflammatory response persists following incomplete removal of the pathogen or even despite its clearance, due to ongoing cross-reactivity of the antibodies to endogenous epitopes. As a consequence, chronic myocarditis/inflammatory cardiomyopathy may develop, progressing to full-fledged DCM [8, 11, 36, 37].

Table 1 Causes of myocarditis: Infectious myocarditis

Viruses	DNA viruses: Adenoviruses, Erythroviruses (Parvovirus B19), Hepatitis B virus, Herpes viruses (Herpes Simplex virus type-1 and type-2, Human Herpesvirus-type {6}, Cytomegalovirus, Epstein-Barr virus, Varicella-Zoster virus), Rabies virus, Variola virus, Vaccinia virus RNA viruses: Chikungunya virus, Enteroviruses (Coxsackieviruses A/B, Echoviruses), Hepatitis C virus, Human Immunodeficiency virus, Influenza A/B viruses, Measles virus, Mumps virus, Polioviruses, Rabies virus, Respiratory syncytial virus, Rubella virus. Others: Dengue virus, Junin virus, Lassa fever virus, Yellow fever virus
Bacteria	*Brucella*, *Chlamydia*, *Clostidrium*, *Corynebacterium diphtheria*, *Haemophilus influenzae*, *Gonococcus*, *Legionella* spp, *Meningococcus*, *Mycobacteria*, *Mycoplasma pneumoniae*, *Pneumococcus*, *Salmonella*, *Staphylococcus*, *Streptococci*, *Vibrio cholera*
Protozoa	*Entamoeba histolytica*, *Leishmania*, *Plasmodium falciparum Trypanosoma cruzi* (Chagas disease), *Toxoplasma gondii*
Spirochaete	*Borrelia burgdorferi* (Lyme disease), *Leptospira* (Weil disease), *Treponema pallidum*
Fungi (uncommon)	*Actinomyces*, *Aspergillus*, *Blastomyces*, *Candida*, *Coccidioides*, *Cryptococcus*, *Histoplasma*, *Mucormycoses*, *Nocardia*, *Sporothrix schenckii*
Parasites and Rickettsia (very rare)	*Echinococcus granulosus*, *Schistosoma*, *Taenia solium*, *Toxocara canis*, *Trichinella spiralis*, *Coxiella burnetii* (Q fever), *Rickettsia rickettsii* (Rocky Mountain spotted fever)

Noninfectious myocarditis

Myocarditis may develop via noninfectious immune-mediated mechanisms, either as isolated cardiac involvement or associated with systemic diseases, which include a wide spectrum of autoimmune and autoinflammatory conditions [38–42], most commonly sarcoidosis, eosinophilic granulomatosis with polyangiitis (EGPA)/Churg-Strauss syndrome,

Table 2 Causes of myocarditis: noninfectious myocarditis

Immune autoimmune	Autoantigens: infectious-negative lymphocytic or giant cell myocarditis (post-infectious or auto-immune inflammatory processes) Associated with immune-mediated or autoimmune/autoinflammatory diseases: Behçet's disease, Churg-Strauss syndrome, coeliac disease, inflammatory bowel disease (Crohn's disease, ulcerative colitis), inflammatory myopathies (dermatomyositis, polimyositis), Kawasaki's disease, sarcoidosis, systemic sclerosis, systemic lupus erythematosus, rheumatic heart disease (rheumatic fever), rheumatoid arthritis, juvenile idiopathic arthritis, ANCA associated vasculitis, non-antibody associated vasculitis including giant cell and Takayasu arteritis Alloantigens: heart transplant rejection Allergens: smallpox and tetanus toxoid vaccinations	
Drugs and toxic substances	Allergic/hypersensitivity reaction (HSM) Psychiatric medications: benzodiazepines, clozapine, lithium, tricyclic antidepressants Antibiotics: ampicillin, azithromycin, cephalosporins ciprofloxacin, isoniazid, penicillin, sulphonamides, tetracyclines Antiphlogistics: mesalamine, phenylbutazone Miscellaneous: adalimumab, colchicine, thiazide diuretics, methyldopa, dobutamine, lidocaine, metoprolol, phenytoin	Direct toxic effects (toxic myocarditis) Antineoplastic drugs: amsacrine, anthracyclines cyclophosphamide, 5-fluorouracil, imatimib mesylate, interferon-alpha, interleukin-2, mitomycin C, mitoxantrone, tyrosine kinase inhibitors (e.g. trastuzumab, anti-HER2/neu) Miscellaneous: amphetamine-derived compounds, antimony compounds, antiretroviral agents, catecholamines (dopamine, dobutamine, norepinephrine, epinephrine), chloramphenicol, chloroquine, hydroxychloroquine, dopamine agonists, emetine, ephedrine lithium, methysergide phenothiazines, phenylpropanolamine, serotonin-derived compounds (fenfluramine/phentermine, appetite suppressants), phenytoin, tricyclic antidepressants, zidovudin Drugs of abuse Alcohol, amphetamines and related compounds (methamphetamines, ecstasy), anabolic steroids, cocaine, opiate overdose
Other recognized causes	Heavy metals: copper, iron, lead, arsenicals Physical agents: radiation Scorpion-bee-wasp stings, snake/spider bite	

systemic lupus erythematosus (SLE), rheumatoid arthritis/juvenile idiopathic arthritis (JIA), scleroderma (systemic sclerosis), myositis and Behçet's disease. Cardiac involvement in these conditions can vary considerably in extent, severity and site (myocardium, pericardium, endocardium, valve tissue and coronary arteries). In autoimmune myocarditis, CD4-positive T-lymphocytes are the main actors [43], but autoantibodies can also directly attack heart tissue and cause myocardial damage [41, 44]. In addition, myocarditis may also be triggered by drugs, vaccines and other noninfectious agents (Table 2) which cause inflammatory reaction leading to cardiac damage and dysfunction. The two most frequent patterns are hypersensitivity myocarditis (HSM) and toxic myocarditis (TM). HSM is considered a consequence of a delayed allergic reaction to the offending drug; in TM, histologic myocyte injury is believed to be a direct toxic effect, not mediated by hypersensitivity. The list of implicated drugs and substances continues to grow (Table 2) [37, 45–47]. Although noninfectious myocardites are thought to be uncommon [45], their correct identification is important due to the substantial morbidity and potential benefit of specific treatments [9].

Pathology of myocarditis

Myocarditis develops within a wider 'inflammatory syndrome' where a complex interplay of pathogen triggers and immune system response occurs. Although in the initial phases evidence of tissue inflammation may be lacking, inflammatory cells are key players of the process and pathologic diagnosis relies largely on their identification and characterization in myocardial tissue. The histopathology spectrum of myocarditis is broad and includes different subtypes with distinctive patterns, reflecting aetiological background. The composition and distribution of inflammatory cells, type and degree of histological myocardial injury and type of anatomical structures involved (myocardial interstitium, myocytes, vessels) offer important etiological clues [48].

Histopathology subtypes and relationship with causes

The current optimum classification of major histopathology subtypes is [37]:

- Lymphocytic myocarditis (LM)
- Giant cell myocarditis (GCM)
- Cardiac sarcoidosis
- Other granulomatous myocarditis
- Eosinophilic myocarditis
- Neutrophilic myocarditis

Inflammatory patterns with mixed or less-defined histopathology features can also be seen as well as pictures with associated vasculitis.

Lymphocytic myocarditis

LM is the commonest histological subtype, characterized by an inflammatory myocardial infiltrate typically comprising mononuclear cells, with a dominant component of T lymphocytes and variable representation of macrophages. A smaller contingent of neutrophils may be present, usually related to the extent of myocyte damage. Plasma cells or eosinophils can be seen, but are usually few: when neutrophils or eosinophils are the preeminent component, other subtypes of myocarditis should be considered [9, 49]. In the acute/active phases, LM is usually accompanied by myocyte damage/necrosis, whose pattern is generally sparse compared to the overall amount of inflammation, although in more advanced and severe cases it can be conspicuous. In subacute/healing stages, myocyte damage is more localized; in healed cases, it may be absent. During the healing process, foci of damaged myocytes decrease and lymphocytes give way to macrophages: mesenchymal reparative tissue appears and is gradually substituted by replacement fibrosis (Fig. 1). The characteristics of inflammatory infiltrates and associated myocyte damage are identical to those of acute cellular rejection in cardiac allografts, whose morphological progression sequence is a useful model to understand the stages of LM [50].

Although not related with a specific aetiology, LM is commonly associated with viruses, even when viral genomes cannot be identified. Alternatively, LM may represent a primary autoimmune condition or the cardiac localization of immune systemic diseases [51–55], such as SLE [56–58], systemic sclerosis [59], inflammatory muscle diseases and rheumatoid arthritis [60, 61]. In these settings, myocyte necrosis associated with inflammation tends to be less evident or minimal; lymphocytic small vessel myocardial vasculitis may be present as can be chronic aspects of small vessel disease.

Giant cell myocarditis

GCM is a severe entity characterized by an extensive, mixed inflammatory infiltrate, principally constituted of macrophages, followed in quantity by lymphocytes and macrophage-derived multinucleated giant cells, typically scattered, with a lesser representation of eosinophils and plasma cells [62] (Fig. 2a–d). This type of myocarditis is generally thought to be autoimmune in nature [63, 64]. It is a distinct clinico-pathologic form, frequently fulminant, and characterized by poor prognosis. Although inflammatory processes with multinucleated giant cells are often referred to as granulomatous myocarditis in autopsy-based literature [65], well-formed granulomas are absent in GCM. Inflammatory cells surround and directly attack

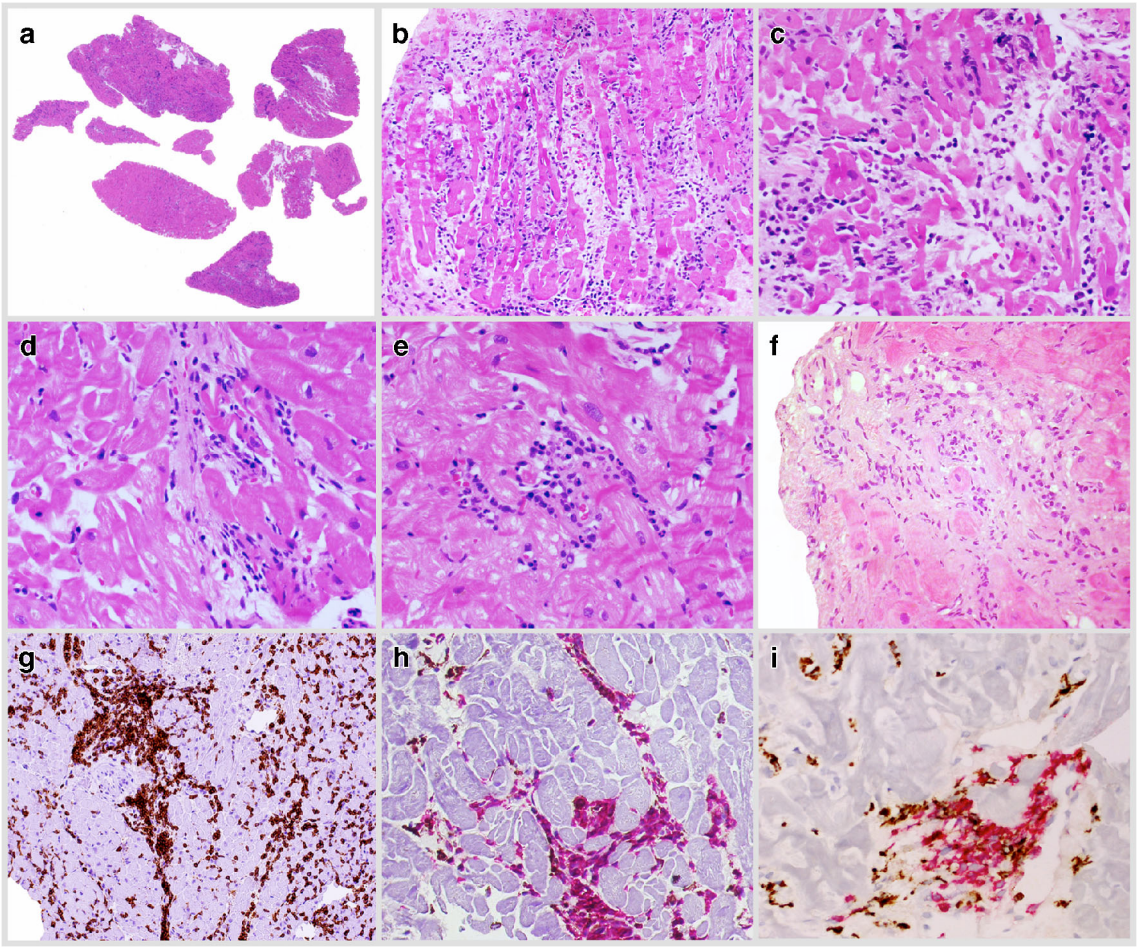

Fig. 1 Aspects of lymphocytic myocarditis. **a**, **b** and **c** Case of diffuse lymphocytic myocarditis in a 45-year male. Severe inflammatory infiltrates throughout all myocardial samples (**a**), associated with oedema and extensive myocyte damage (**b**–**c**). Inflammation is mainly composed of T-lymphocytes with a minor component of macrophages; neutrophils are attracted by myocyte necrosis. Haematoxylin-eosin, original magnification: **a**: × 25x; **b**: × 200; c: 400. **d**–**e**. Two cases of focal active lymphocytic myocarditis (40-year female; 20-year male): lymphocytic infiltrate is limited but is anyway associated with myocyte damage. Haematoxylin-eosin, original magnification × 400. f. Biopsy control in a 30-year female after previous histologic diagnosis of multifocal lymphocytic myocarditis. This is healing myocarditis, where lymphocytic inflammation is reduced and mainly localized within the mesenchymal/fibrous reparative tissue. Limited myocyte damage is still focally present. Haematoxylin-eosin, original magnification × 200. **g** CD3 immunostaining highlights that inflammatory infiltrates are mainly composed of T lymphocytes. Original magnification: × 200. **h**–**i**. Double CD68/CD3 immunostaining can help to distinguish and quantify T-lymphocytes (in red) and macrophages (in brown). Original magnification: × 400.

the myocytes and, in the early phases, myocardial damage is extensive and multifocal, as has been observed in cases of SCD. As the disease progresses towards chronicity, inflammation is less diffuse and severe, giant cells become rare, myocyte damage becomes focal and reparative fibrosis appears.

Cardiac sarcoidosis

Cardiac sarcoidosis, like giant cell myocarditis, is characterized by unique histopathologic features generally allowing a diagnosis of certainty by the pathologists, although the histological interpretation must always follow clinical contextualization. Histological findings are fully superimposable to those of extracardiac sarcoid disease, epitomized by well-formed, separate, epithelioid granulomas, usually with giant cells, surrounded by T lymphocytes, the latter abundant in early phases. Granulomas are characteristically non-necrotizing; giant cells may contain Schaumann and asteroid bodies, although these are not specific for sarcoidosis (Fig. 2e–h). Foci of exclusively lymphocytic myocarditis can be observed, separate from granulomatous areas, and can cause misdiagnosis of LM by EMB due to random sampling. Myocyte necrosis is absent in the proximity of granulomas, but can be seen in association with the lymphocytic infiltrates. Granulomas are surrounded by variable degrees of interstitial fibrosis, which originates at the periphery and extends over time due to deficiencies in immune regulation and inflammatory and profibrotic genetic signatures. In late lesions, large sclerotic areas with confluent granulomas are present and set the stage for end-stage sarcoidosis [66, 67].

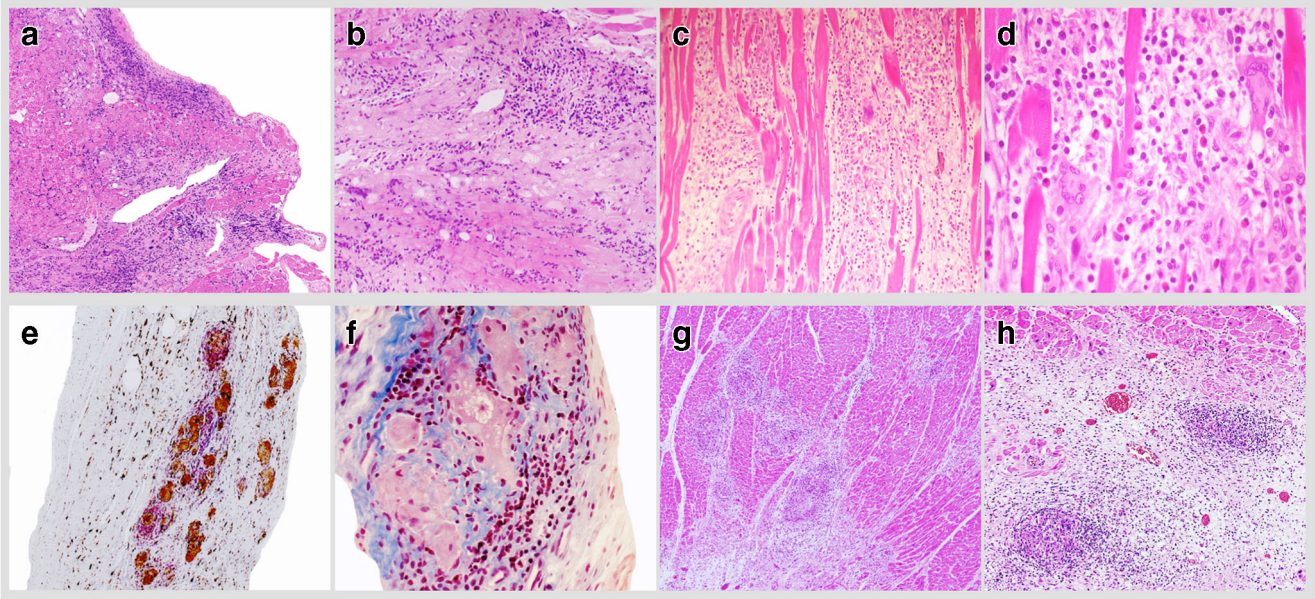

Fig. 2 Giant cell myocarditis and cardiac sarcoidosis. **a**–**d** Giant cell myocarditis. EMB of a 34-year male (**a**–**b** Haematoxylin-eosin, × 100; × 200); sudden death of a 21-year-old male (**c**–**d** Haematoxylin-eosin, × 100; × 200). Inflammation is typically widespread and mixed (macrophages, lymphocytes, a variable component of eosinophils and plasma cells and a substantial number of scattered multinucleated giant cells) and causes significant myocyte damage. There are no clearly formed granulomas. **e**–**h** Cardiac sarcoidosis. **e**–**f** EMB of a 47-year female with isolated cardiac sarcoidosis. **e** Double CD68/CD3 immunostaining (× 50) shows macrophages in brown and T lymphocytes in red; **f** Mallory trichrome, × 400. **g**–**h** Case of systemic sarcoidosis with cardiac involvement in sudden death of a 32-year-old female (Haematoxylin-eosin, × 50; × 100). In both cases, there are numerous well-formed epithelioid granulomata with giant cells, surrounded by T lymphocytes and, partially, by fibrous tissue. Myocyte damage is usually not significant near granulomata. The degree of fibrosis depends on stage of the disease.

Although sarcoidosis has been described nearly 90 years ago [68], its aetiology remains largely unknown. Hypotheses include a mix of genetic, environmental, infectious and immune factors [69]. Cardiac involvement is generally part of systemic multi-organ/multi-tissue disease, although isolated cardiac sarcoidosis has been described [70]: its incidence is highly variable ranging from 4 to 5% in clinical series to 20–25% in autopsy studies [69]. Clinically, cardiac sarcoidosis is difficult to suspect and frequently missed, as explanted hearts and EMB studies show [71]. This is unfortunate, because advanced structural involvement leads to intractable heart failure and/or refractory arrhythmias which may require heart transplantation [69–72].

Other granulomatous myocarditis

Other myocarditis with histologic granulomatous features may be related to infections, mainly tuberculosis, other mycobacterial forms or fungi, and can be found in chronic cardiac Chagas disease or other parasitic infections. Histologic patterns of granulomas (caseating, necrotizing, suppurative) can help in differential diagnosis, but staining for acid-fast bacilli (Ziehl Neelsen), fungi (PAS and Grocott) and parasites (Giemsa) should always be performed. As always, all histopatologic findings must be interpreted in the broader clinical context [48].

Eosinophilic myocarditis

Eosinophilic myocarditis is a rare variant described in association with a wide spectrum of diseases/conditions classified by cause: hypersensitivity reactions; immune-mediated diseases, such as EGPA (former Churg Strauss syndrome); idiopathic hypereosinophilic syndrome (HES); non-haematologic malignancies; parasitic infections and drugs/vaccines. It may or may not be associated with peripheral blood eosinophilia [73]. The common denominator is the eosinophilic infiltrate, which may represent the major inflammatory component, depending on the underlying disease or type of stimulus, or be part of a complex picture of mixed inflammation with lymphocytes, macrophages, plasma cells, poorly formed microgranulomas and giant cells [74]. Clinical suspicion of eosinophilic myocarditis followed by EMB is essential in these forms in order to allow specific treatments including, when fulminant, mechanical circulatory support [5, 75].

Besides Loeffler endomyocarditis, a subcategory of HES in which the heart is predominantly involved (Fig. 3), the two most common forms appear to be drug-related HSM and EGPA-myocarditis [76]. HSM is probably the most prevalent expression of drug-induced myocarditis (Table 2) consequent to a delayed allergic reaction to the offending drug: it is dose-independent and usually reversible after cessation of the causative agent [47, 77].

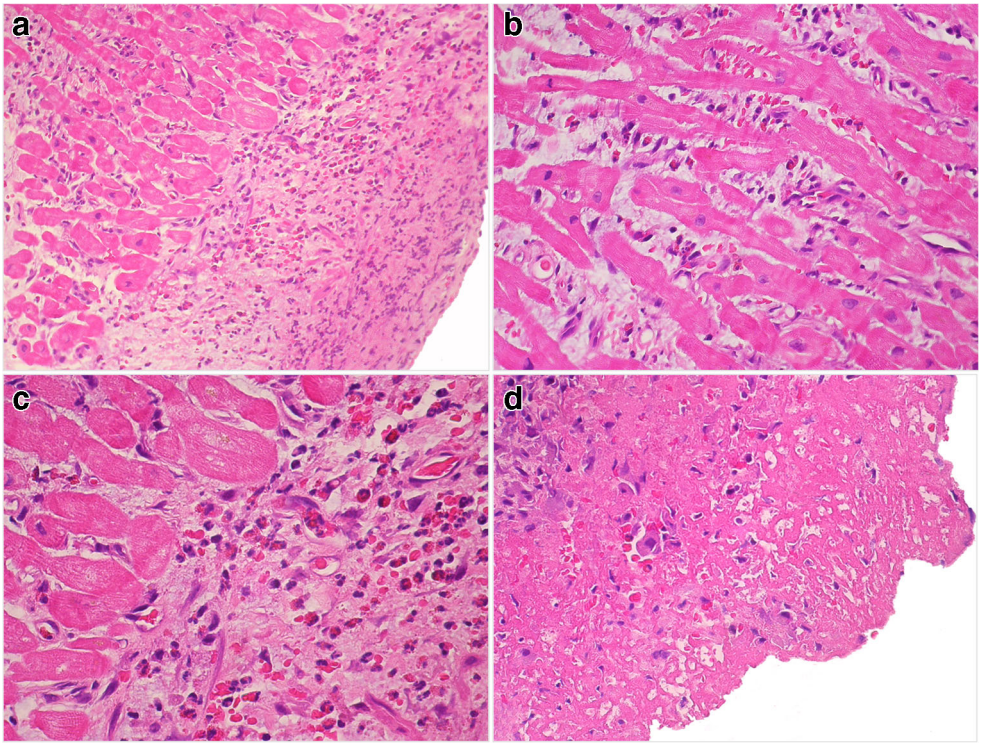

Fig. 3 Early phase of non-tropical eosinophilic endomyocardial fibrosis (Loeffler endomyocarditis) in a 43-year-old male with primary hypereosinophilic syndrome (white blood cells 38.000; eosinophils 38%). **a**–**d**: EMB shows eosinophilic myocarditis with endomyocardial infiltration by eosinophils, degranulated or not, together with some lymphocytes and macrophages (**a**–**c** Haematoxylin-eosin, × 200, × 400, × 400), and initial thrombotic deposition on the endocardium surface (**d** Haematoxylin-eosin × 400). Two years previously, the patient was operated on for mitral and aortic valve replacement due to nonbacterial thrombotic endocarditis

The general incidence of HSM varies from autopsy data (2–5%) to explanted hearts of patients awaiting transplantation and receiving multiple drugs for advanced heart failure (2.7 up to 23%) [47, 78, 79]. Its clinical spectrum ranges from asymptomatic/subclinical forms, usually diagnosed at explant or autopsy, to fulminant, life-threatening forms [5, 75]. The HSM pathology spectrum is ample: interstitial inflammatory infiltrates may range from limited to dense and widespread, usually depending on whether the disease is symptomatic or found incidentally; myocyte necrosis may be absent, focal/multifocal or atypically extensive. Eosinophil-rich HSM with small, poorly formed macrophage-granulomas and no or very focal myocyte necrosis can be observed in patients on multiple drug therapy awaiting transplantation, while a rather different pattern occurs in patients suffering from ulcerative colitis treated with mesalamine (Fig. 4).

The histology pattern of cardiac involvement in EGPA is complex and shows several aspects of overlap with HSM. In the active phases, vasculitis is typically associated with myocardial inflammation. Finally, necrotizing eosinophilic myocarditis is the most severe and aggressive form in this group, characterized by extensive myocardial necrosis and diffuse interstitial inflammation composed mainly of eosinophils and macrophages, scattered lymphocytes and plasma cells. Its pathogenesis is thought to result from eosinophil degranulation with release of granule cytotoxic proteins, which induce necrosis of the myocardium and inflammatory cells [80–82].

Neutrophilic myocarditis

Neutrophilic myocarditis is an uncommon histological pattern typical of bacterial myocarditis, generally observed in immunocompromised individuals. Neutrophils are the major component of the inflammatory infiltrates and can be patchily distributed around myocytes or aggregated in micro-abscesses. Significant myocyte damage is usually associated. Numerous bacteria can be implicated and myocardial involvement is more frequently due to haematogenous dissemination in the course of septicaemia. Neutrophilic myocarditis can result from diffusion of extensive bacterial pneumonia or, rarely, complicate bacterial endocarditis [83]. Neutrophilic microabscesses with abundant myocardial necrosis or necrotizing inflammation may also be found in fungal myocarditis (Fig. 5), occasionally coexisting with granulomas and giant cells [48]. In rare instances, bacterial myocarditis can take other forms: granulomatous inflammation, in tuberculosis and Whipple disease; lympho-histiocytic infiltrates with a variable degree of neutrophils, in leptospirosis (Weil disease) and a nonspecific LM, in Lyme disease, where direct Borrelia Burgdoferi toxicity is thought to intervene with or without a coexisting immune-mediated component [48]. In the acute phase of Chagas disease, dense inflammatory infiltrates with lymphocytes, plasma cells, macrophages and possible rich component of neutrophils are associated with myocyte necrosis [48].

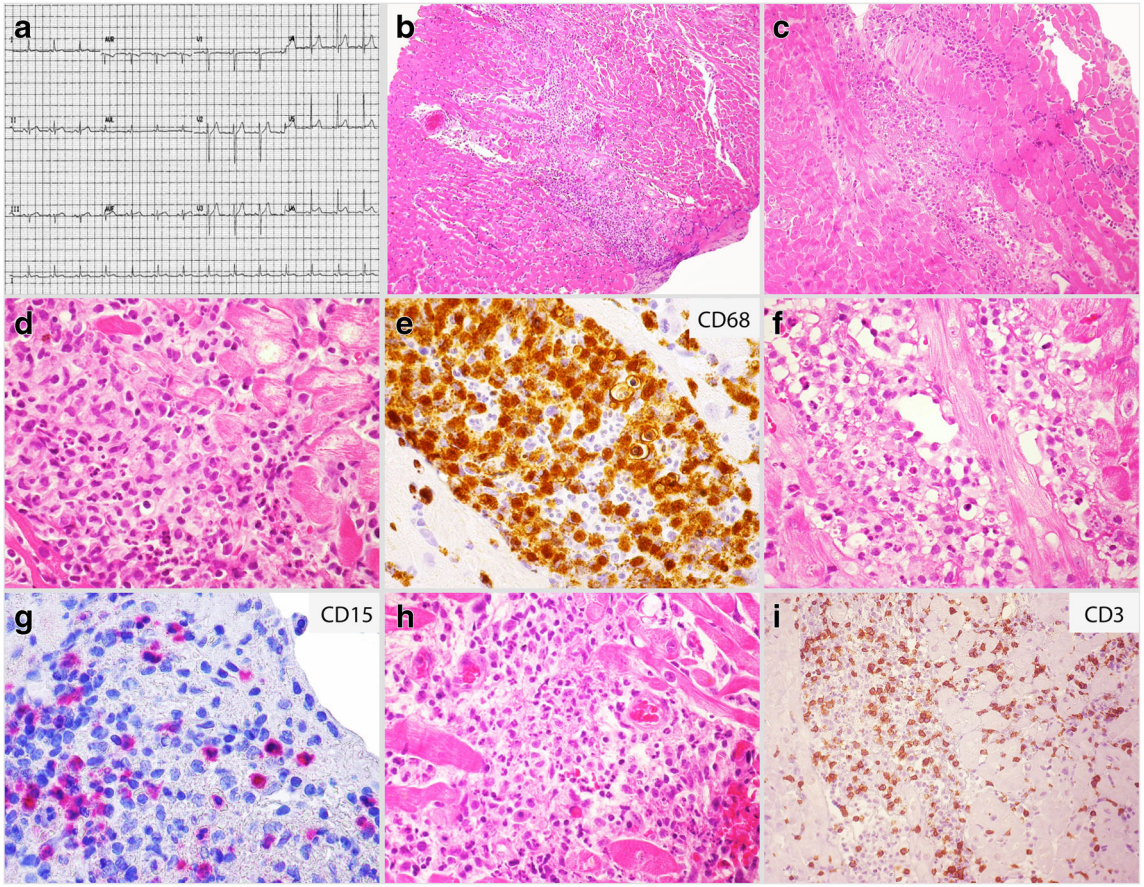

Fig. 4 Hypersensitivity myocarditis. A 28-year-old male in therapy with mesalamine for recurrent ulcerative colitis was hospitalized with oppressive chest pain. Troponin level was elevated (0.06 mg/ml); electrocardiogram showed pericarditis/perimyocarditis-like features (sinus tachycardia and diffuse ST-elevation with upward concavity without reciprocal changes) (**a**); echocardiogram revealed mildly depressed left ventricle systolic function with ejection fraction of 45–50% and mild pericardial effusion. EMB histology showed multifocal micro-granulomatous myocarditis with mixed inflammation (**b**–**c**, Haematoxylin-eosin, × 100) of many macrophages (**d** Haematoxylin-eosin, × 400; **e** CD68 immunostaining, × 200), a significant number of eosinophils (**f** Haematoxylin-eosin; **g** CD15 immunostaining; × 400), and some neutrophils (**h** Haematoxylin-eosin, × 400) and lymphocytes (**i** CD3 immunostaining × 200), associated with multifocal myocyte damage. After mesalamine withdrawal and graduated doses of prednisone, azathioprine (50 mg 2 cp/die) and ciprofloxacin (500 mg 2 cp/die for 6 days), the patient's condition improved with progressive drops in cardiac biomarkers and improvement in left ventricular systolic function. The specific mechanism for mesalamine-induced cardiovascular toxicity is not completely understood but is thought to be humoral-mediated hypersensitivity, where antibodies formed against mesalamine cross-react with cardiac tissue, causing inflammation

Histopathologic variants difficult to classify

Lympho-histiocytic myocarditis

Some forms of myocarditis show a lympho-histiocytic histopathologic pattern where both lymphocytes and macrophages are present, but the latter are more significant than in LM, and may represent the prevalent cell population. This pattern may be related to healing LM; unusual forms of viral myocarditis; macrophage-rich autoimmune diseases, such as juvenile idiopathic arthritis (Fig. 6) and HSM or advanced stages of GCM. The term is rarely used today, because it is ambiguous to clinicians and discouraged by pathologists. It was used in the past for granulomatous myocarditis, especially when macrophage aggregates had a vaguely granulomatous appearance [37]. Nevertheless, a lympho-histiocytic pattern is increasingly recognized—due to a more diffuse use of immunohistochemical typing—as macrophages play a prominent role in inflammatory heart conditions such as antibody-mediated or mixed type rejection [84, 85].

Myocarditis with vasculitis and microvascular inflammation

Some inflammatory myocardial diseases can have distinctive aspects of cardiac microvasculature vasculitis. In general, this pattern is part of the picture of

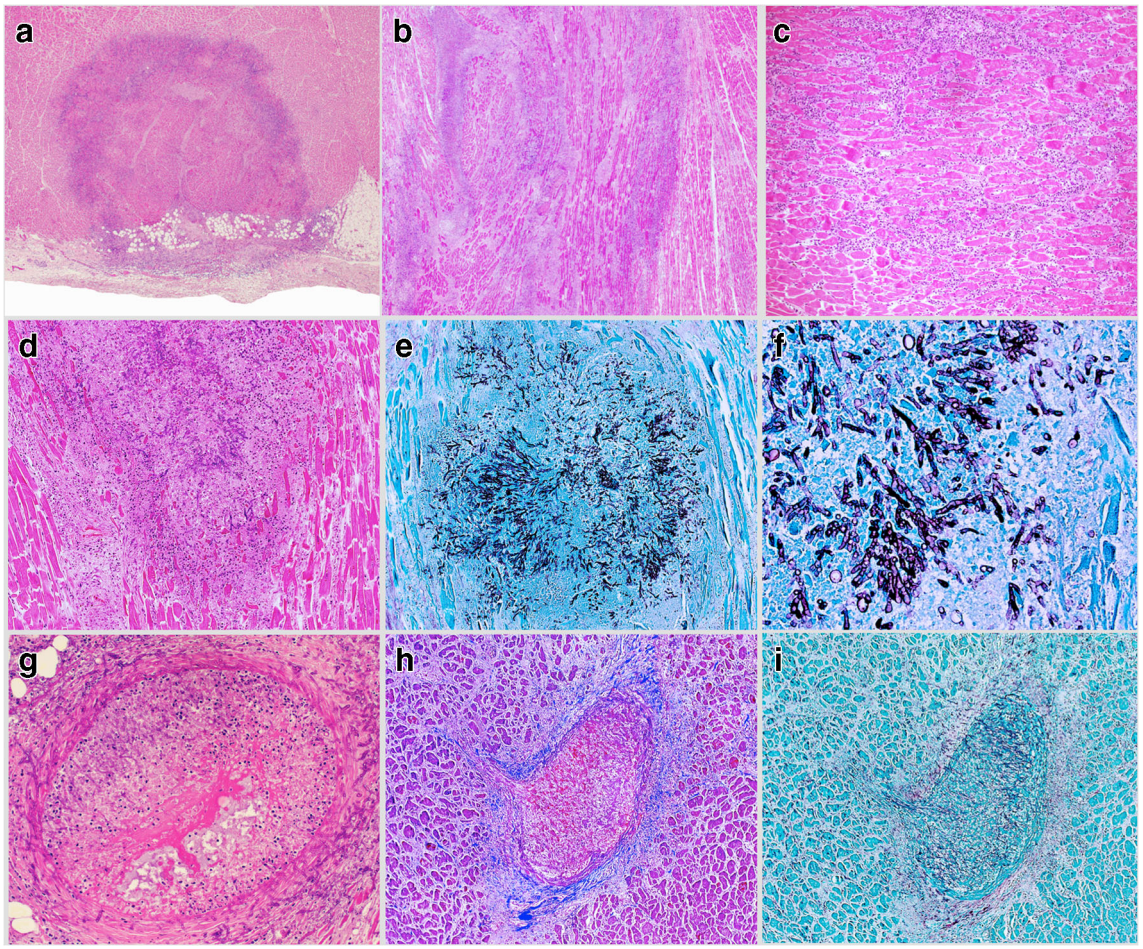

Fig. 5 Aspergillus necrotizing myocarditis. Fifty-four-year male affected by systemic lupus erythematosus treated over a period of 10 years with cycles of immunosuppressive therapy (steroid and rituximab). The patient died due to disseminated invasive aspergillosis with multiorgan involvement (heart, lungs, brain, kidneys, thyroid, pancreas, adrenal glands). **a**–**i** In the heart, extensive necrotizing myocarditis was found irregularly distributed throughout the myocardium in subepicardial (**a** Haematoxylin-eosin, × 25) midmural (**b** Haematoxylin-eosin, × 25) and subendocardial areas. Myocarditis foci consisted of neutrophilic infiltrates (c, Haematoxylin-eosin, 100x) mixed with fungal hyphae of *Aspergillus fumigatus* (**d** Haematoxylin-eosin, × 100), in black with Grocott staining (**e**, × 100; **f**, × 400), and associated with abundant myocyte necrosis. Diffuse septic thrombi were present in coronary arteries (**g**, Haematoxylin-eosin, × 200; **h**, Mallory trichrome, × 100; **i**, Grocott staining, × 100)

myocarditis associated with systemic autoimmune disorders such as SLE, rheumatoid arthritis, polyarteritis nodosa and EGPA.

The histologic pictures may on occasion overlap with those of lymphocytic, lympho-histiocytic and eosinophilic myocardites, and in such cases, it is far from simple to reach a conclusive diagnosis. A detailed description of vasculitis involving the myocardium would go beyond the scope of this review.

It should, however, be mentioned that the histologic features of antibody-mediated rejection (microvascular inflammation characterized by endothelial cell swelling and intravascular mononuclear cells) can serve as a histopathology model to throw light on some forms of myocardial vasculitis and the underlying immune mechanisms [84, 85].

Histopathology of toxic myocarditis

Toxic myocarditis is an aetiologic denomination indicating direct myocardial injury by numerous drugs or substances, whether part of medical treatment or illicit (Table 2). It is dose-dependent, has a possibly cumulative effect and persists after drug cessation [46, 48]. Although variable and non-specific, the histologic features of toxic myocarditis consist of two main patterns: an early stage with microfoci of solely necrotic/damaged myocytes and the later phase of 'myocarditis' (Fig. 7). Toxic myocarditis frequently indicates inflammatory stages of catecholamine-induced myocardial injury. Catecholamine toxicity on the heart has been known since the 1960s–70s in patients with pheochromocytoma. More recently, these lesions have been found in brain-death donor hearts rejected for transplantation, secondary to

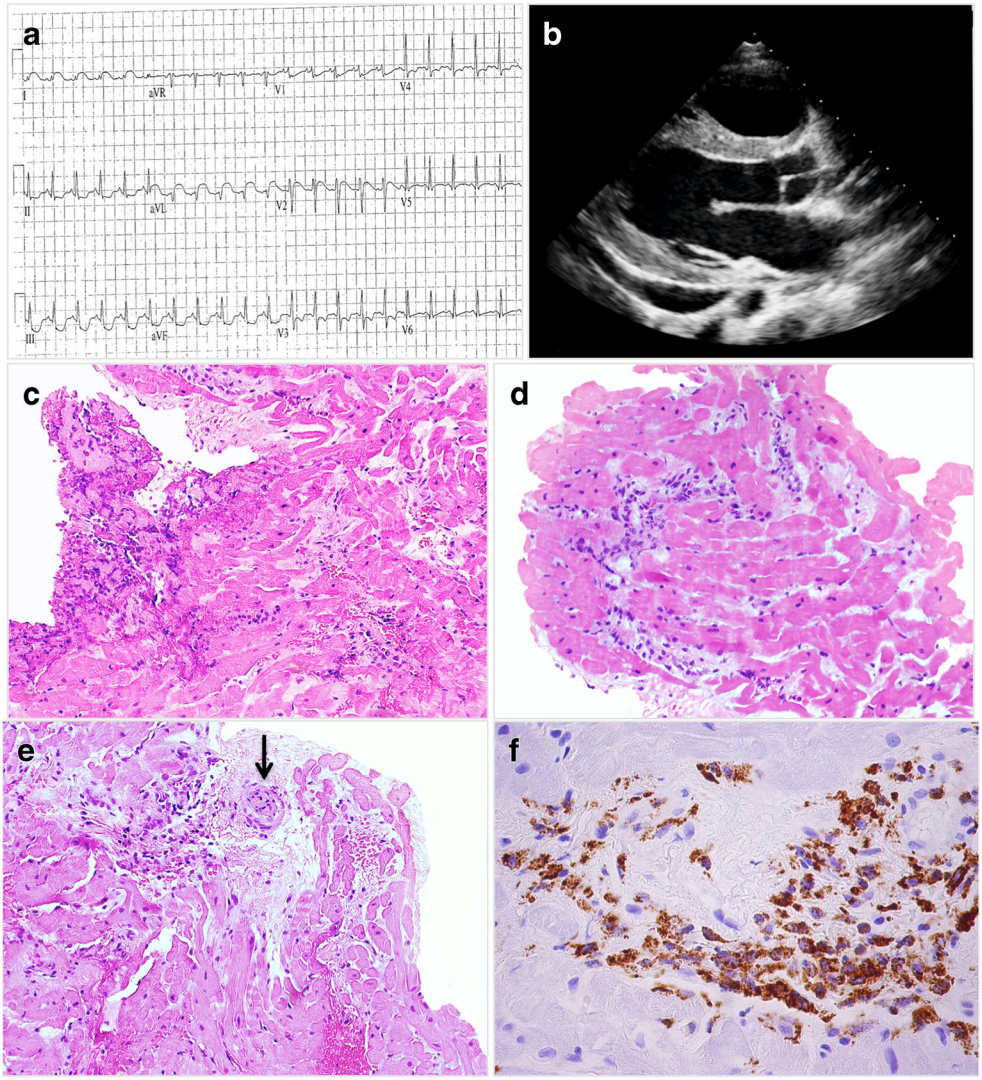

Fig. 6 Myocarditis with prevalent macrophage inflammation. Myocarditis in a 17-year-old male where final diagnosis was: adult-onset juvenile idiopathic arthritis with active systemic features and without active arthritis. The patient was admitted to the coronary unit with fever and chest pain. ECG showed a pattern of diffuse ST elevation (**a**); at echocardiography, there was severe reduction in ejection fraction and pericardial effusion (**b**). **c**–**f** EMB showed active multifocal myocarditis predominantly constituted by macrophages, in some areas mixed with fibrin and platelets, with a minor component of granulocytes and T lymphocytes (**c**–**d** Haematoxylin-eosin, × 200). Microthrombi of fibrin and granulocytes were also present in some small vessels (arrow) (**e** Haematoxylin-eosin, × 200; **f** CD68 immunostaining for macrophages, × 400). PCR for viral genomes on myocardial tissue and blood was negative as were other laboratory tests for serum immunologic assessment and bacterial or viral infections. Therapy with steroids and methotrexate resulted in rapid and complete resolution of symptoms

catecholamine-release during the 'sympathetic storm' following brain-death or administered as pharmacologic support [86–88]. In the Bologna heart transplant centre, the wide spectrum of these toxic lesions has been studied in detail in routine pathology examination of donor hearts unsuitable for transplantation [86].

Special mention must be made of autoimmune myocarditis which can develop after checkpoint inhibitor therapy. Here, the histopathology differs from that of other toxic myocarditis, as it consists of T cell-predominant lymphocytic infiltrates within the myocardium [89]. This novel class of anticancer drugs has revolutionized the management of many malignancies with poor prognosis, but its cardiac toxicity has been largely underestimated. Toxic myocarditis in these patients has turned out to be more frequent and severe than expected, although it may respond to steroids [89, 90].

Diagnosing myocarditis by EMB

Pathologists can diagnose myocarditis in various settings: at post-mortem, either as the only cause or concurrent cause of

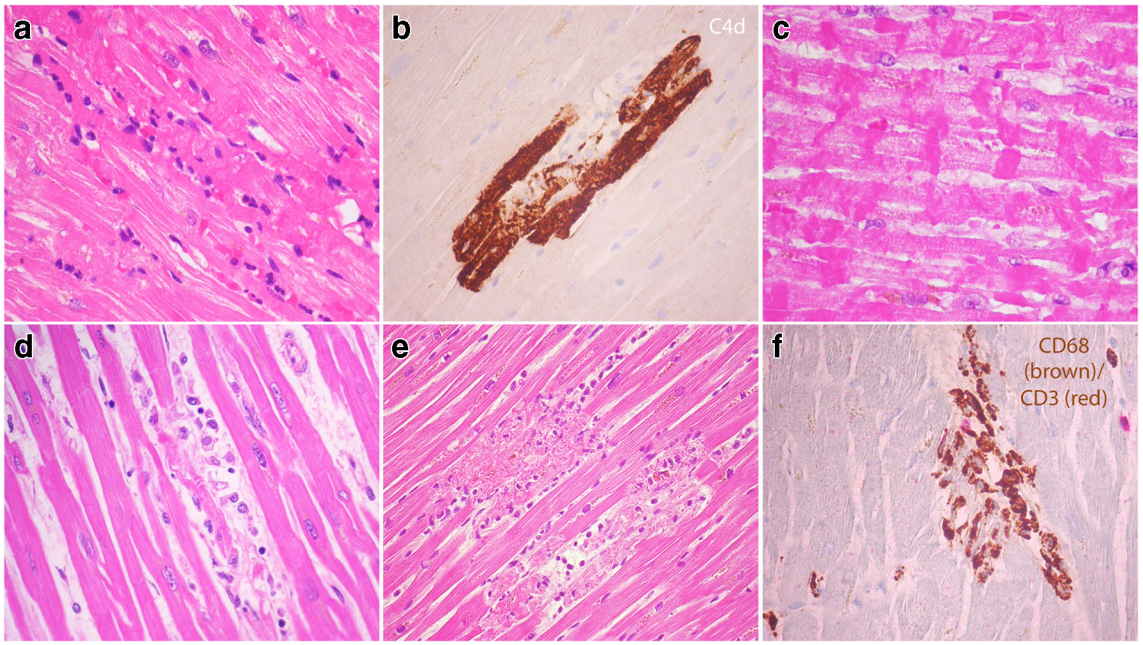

Fig. 7 Histology of toxic myocarditis. **a**–**c** Early stage with microfoci of necrotic/damaged myocytes: myofibre eosinophilic changes (**a** Haematoxylin-eosin, × 400), vacuolar degeneration, coagulative necrosis (**b** C4d immunostaining, × 400), prominent contraction band necrosis (**c** Haematoxylin-eosin, × 400). **d**–**f** Phase of 'myocarditis': multiple groups of different stages of dead cells (hypereosinophilic, disrupted, fragmented, necrotic myocytes) surrounded by sparse inflammatory infiltrates (**d**–**e** haematoxylin-eosin, × 200) primarily composed of macrophages, occasional neutrophils and a small number of lymphocytes (**f** double CD68/CD3 immunostaining, × 200)

SCD, or as an occasional finding during routine autopsy; when examining an explanted heart and when EMB is performed. These very different situations often imply different types of myocarditis with diverse extent and evolution. Today, EMB is the main tool for in vivo diagnosis of myocarditis but, as an invasive procedure, its use is limited to selected patients. Its unquestionable diagnostic value needs to be weighed against the (limited) procedural risk and (more relevant) potential lack of sensitivity, particularly when untargeted biopsies are performed in multi−/microfocal disease.

Cardiac EMB is invaluable under many aspects: it is the gold standard for the definitive diagnosis of myocarditis by identifying the inflammatory infiltrates in myocardial tissue, allowing histologic subtype classification and directing towards the most likely aetiology (infective, immune, drug-related, specific forms), when combined with immunohistochemistry and molecular studies [91, 92], thus playing a decisive role in management and prognostication. Furthermore, EMB provides an estimate of the extent and degree of activity of the inflammatory process, allowing accurate staging of myocarditis. Even when the aetiological diagnosis of myocarditis is known, e.g. in the context of systemic inflammatory disease, EMB can provide vital information for clinical decision-making. The EMB protocol begins with histopathological assessment, the essential initial step to evaluate inflammatory patterns, identify disease subtype and point to likely aetiopathogenesis. In a comprehensive, contemporary protocol for EMB analysis, histopathology must be followed and supported by a combination of histochemistry, immunohistochemistry and molecular studies such as in situ hybridization and PCR for viral genomes, based on the clinical context, in order to complete the initial picture.

Histopathology

After more than three decades, the Dallas Criteria still stand as the histologic cornerstone for the diagnosis of myocarditis in EMB specimens [93], which requires the presence of 'inflammatory infiltrates of the myocardium with necrosis and/or degeneration of adjacent myocytes, not typical of ischemic damage associated with coronary artery disease'. Following the identification of inflammatory infiltrates, their composition, distribution and extent, as well as the presence, pattern and amount of fibrosis, should be carefully detailed (Table 3). Myocyte damage includes a spectrum of heterogeneous and subtle features: clearing/vacuolisation or fragmentation/disruption of myocytes, myocytolysis, inflammatory encroachment and necrosis. All these

Table 3 Dallas Criteria: morphological diagnosis of myocarditis

Classification	
First biopsy ■ Myocarditis with or without fibrosis ■ Borderline myocarditis (repeat biopsy may be indicated) ■ No myocarditis	Subsequent biopsy ■ Ongoing (persistent) myocarditis with or without fibrosis ■ Resolving (healing) myocarditis with or without fibrosis ■ Resolved (healed) myocarditis with or without fibrosis
Descriptors	
Inflammatory infiltrates Distribution: focal, confluent, diffuse Extent: mild, moderate, severe Type: lymphocytic, eosinophilic, granulomatous, giant cell, neutrophilic, mixed	Fibrosis Distribution: endocardial, interstitial Extent: mild, moderate, severe Type: perivascular, replacement

aspects are similar to those seen in myocardial cell-mediated rejection [94].

Despite their enduring value in clinical practice, the Dallas Criteria show increasing limitations in light of evolving knowledge [91, 95, 96], especially due to their limited ability in differentiating various forms of myocarditis and interpret their chronic/healing stages [37, 49, 95]. The time seems ripe for a quantic step forward in the field. Ideally, the guiding principles of a revision of the Dallas criteria should be to improve the relation of various histopathologic patterns to specific aetiologies and include histologic diagnostic criteria for the definition of healing/resolution and a description of chronic forms of myocarditis. Integration of histopathologic descriptors, various aspects of myocardial damage and immunohistochemical information could also be extremely useful here.

This would allow closer correlation with clinical pictures in order to predict outcome and promote individualized management.

Immunohistochemical and molecular pathology analysis of myocardial tissue

Immunohistochemistry

Immunohistochemistry (IHC) has proven useful to identify and characterize inflammatory infiltrates in myocarditis [97], enhancing the sensitivity of EMB [9, 98, 99]. Historically, IHC has been instrumental in establishing a cut-off for inflamed versus non-inflamed myocardium [100–105]; in improving recognition of small numbers of sparse inflammatory cells in chronic forms [23, 97, 106–108] and in decreasing inter-observer variability when adjudicating inflammatory cells [109]. These considerations led the European Society of Cardiology (ESC) to include IHC as a criterion for definition and diagnosis of myocarditis: *≥ 14 leukocytes/mm2 in the myocardium, including up to 4 monocytes/mm2, with the presence of CD3 positive T lymphocytes > 7 cells/mm2* are considered an acceptable cut-off for defining an inflammatory infiltrate as abnormal [4]. Including quantitative IHC criteria helps recognition of subacute or chronic myocarditis and provides a guide to identify resolving/resolved forms and borderline myocarditis with minor myocyte damage; these are frequently related to the evolution of LM—whether following persistence or clearance of the virus—towards the late autoimmune stage [12, 36, 110]. IHC increases the yield of EMB for myocarditis and helps predict adverse outcome including cardiovascular death and heart transplantation [99]. In addition, reliable IHC markers of immune activation can assist in evaluating the level of activity of myocarditis and identify phases in which inflammatory infiltrates are not yet visible [111]. IHC for humoral immune response may elucidate other contributing mechanisms involved in myocyte necrosis, fibrosis and remodelling [8], such as antibody-mediated rejection in heart transplantation [84]. In a clinical context strongly indicative of myocarditis, special attention should be paid to atypical histopathologic patterns, where myocyte necrosis/damage is associated with scant inflammatory infiltrate but accompanied by tissue expression of immune-mediated injury [112]. Pathologists still need to develop standardized parameters to assess and classify these patterns [113].

Molecular analysis

Despite well-recognized limitations and interpretative issues, molecular testing for infectious agents has become part of routine EMB-based diagnostics. Current EMB protocols use molecular amplification methods, such as PCR,

reverse-transcriptase-PCR and nested PCR, to detect cardiotropic viruses in lymphocytic myocarditis, both in active histological patterns and in chronic quiescent/resolution stages [52, 92, 114, 115]. Current molecular diagnostic protocols recommend checking for the following viruses: Enteroviruses, Adenoviruses (especially in children), Cytomegalovirus, Epstein–Barr virus, Herpes Simplex virus 1 and 2, Human Herpesvirus 6, Parvovirus B19, Influenza viruses A and B, and Hepatitis C Virus in HCV+ patients [19, 23, 28, 91, 116–118]. However, identifying a viral genome in myocardial tissue does not necessarily mean that a virus is the causative agent of myocarditis [119], nor does a negative PCR exclude the hypothesis of viral disease [120–122]. A parallel molecular investigation of EMB tissue and peripheral blood, together with quantitative evaluation of virus load, is essential to evaluate the presence of systemic infections [4, 92]. Although not routinely performed, investigation of infective status in EMB specimens, i.e. by identifying replicating forms of viral genomes, should be regarded as the gold standard [37, 120–122]. In the rarer forms of bacterial, fungal and parasitic myocarditis, the infectious agents can be identified by histology and special stains (Grocott, Periodic acid Schiff, Giemsa, Gram, Ziehl-Neelsen) and then confirmed by molecular analysis. An important caveat is that all molecular analyses must be interpreted by expert professionals, ideally with the help of a microbiologist: virus incidence in the community, issues of latent infection or viral integration in the host cells with possible reactivation, degree of infectivity and pathogenicity of the virus and patent immunocompetence are all important aspects to be considered before reaching a final aetiological diagnosis.

Future directions—transcriptomics

Transcriptome-based analysis of EMB is likely to bring key contributions to the study of inflammatory myocardial diseases in the near future [123, 124] and help broaden our definition of myocarditis [11]. Myocardial rejection in heart transplantation is again a good paradigm in such perspective [125, 126]. Microarray analysis can be easily applied to EMB to identify the molecular fingerprint of inflammation not visible at histology and recognize very early stages of myocarditis, or characterize unusual histological findings [126, 127]. Molecular signatures may allow the identification of particular phenotypes related to individual sets of genes, mRNA transcripts and proteins. As with any other laboratory technique, microarray study results should be interpreted in the context with the available clinical, laboratory and instrumental diagnostic tools: integration of molecular profiles with histopathologic patterns is the winning approach to improve diagnostic accuracy in myocarditis [128].

Limitations of EMB and role of a myocarditis 'heart team'

The most critical limit to EMB diagnostic accuracy is sampling error leading to false-negative results [129–132]. Although myocarditis generally occurs as a diffuse process, the type, aggressiveness and time-course all can influence the distribution and intensity of inflammation, from dense, widespread infiltrates to small, focal and patchy aggregates. Shield et al. showed that right ventricular (RV) EMB sensitivity is high in patients with GCM (approximately 80 to 93%), particularly when biopsied early and in the presence of a fulminant clinical course, compared with the low diagnostic yield of EMB in sarcoidosis (25%) and lymphocytic myocarditis (35%) [133–135]. Greater sensitivity is also expected in other aggressive forms such as acute necrotizing eosinophilic myocarditis [76].

The number of samples is the major factor influencing diagnostic yield: post-mortem studies performed in the late 1980s showed that EMB sensitivity for myocarditis increases from 17 to 20% with 1 myocardial fragment to almost 80% with 17 specimens: the usual sampling of 4–5 biopsies identified myocarditis in about 50% of cases [132, 136]. Thus, repeating biopsies in patients with strong suspicion of myocarditis may offer the opportunity to increase EMB performance [132, 137]. Establishing in five–six specimens as a norm and modifying their number according to clinical setting (fulminant/non fulminant forms, expected focal myocarditis) is probably the best approach. The other essential point is serial sectioning of specimens, which allows examination of multiple histologic levels and increases the possibility of identifying inflammatory infiltrates, their variety and morphology [138].

Another important step to optimize EMB diagnostic accuracy is to establish a partnership of pathologists and clinicians who are adequately trained for an integrated multidisciplinary interpretation. The pathologist must have specific training in EMB diagnostics, combining an advanced level of expertise in handling and interpretation of biopsy specimens with in-depth knowledge of the associated clinical issues [92, 139]. Appropriate, standardized processing and preparation of specimens and contemporary multistep diagnostic protocols must be implemented, including targeted use of the histopathologic and molecular techniques outlined previously (Table 4). The clinician must be able to perform EMB safely, and to carefully evaluate the timing of biopsies; to judge when to prefer left ventricular (LV) or biventricular EMB to the customary RV-EMB and when to resort to cMRI or other techniques to guide EMB, such as electroanatomic mapping in patients with suspected myocarditis presenting with ventricular arrhythmias [76, 140–149].

Table 4 Requirements for optimal EMB pathologic analysis

Number of specimens and fixation	At least 5 endomyocardial fragments, each 1–2 mm in size, immediately fixed in 10% buffered formalin for light microscopic examination If focal myocarditis is expected, additional sampling is recommended One or two specimens for possible molecular tests. Depending on Centre practice they should be: • Snap frozen in liquid nitrogen and then stored at − 80 °C • Stored in RNA-later (a solution preventing RNA degradation) at room temperature • Sent without any fixative in Eppendorf tubes, immersed in ice
Processing	Use rapid procedure: • Tissue processor with fast biopsy preparation programme • Laboratory microwave oven • Vacuum-prepared automatic processor
Preparation (cutting)	There is no consensus on practice but, as for EMB protocol for rejection monitoring in heart transplantation, it is necessary to: • Serial, numbered sections from the paraffin block (optimally to a max of 60 sections), using at least two thirds of the samples, should be cut in order to examine multiple histologic levels • The number of sections on each slide may vary from 3 to 6 • Alternate sections (around 30) should be stained with Haematoxylin–Eosin • Intermediate unstained sections should be reserved for possible special stains and immunohistochemistry If myocarditis is focal, further serial numbered sections should be cut and alternate sections stained, as above
Diagnostic multistep protocol	
Histology	The pathologist should assess all the morphologic findings described in the paper in order to target the aetiology, when possible
Special stains	Masson or Mallory trichrome: to assess fibrosis, myocyte damage and vessel morphology PAS with and without diastase, Grocott: for fungal infections ZN: for mycobacterial infections Gram: for bacterial infections
Immunohistochemistry	Although there is no common practice among Centres, CD3, CD20, CD4, CD8, CD68 (PGM-1/KP-1) and HLA-DR should be used The immunohistochemistry laboratory must use quality control
Molecular tests	Harvested heart specimens can be used to detect nucleic acids of Enteroviruses, Adenoviruses, Cytomegalovirus, Epstein-Barr virus, Human Herpesvirus 6, Herpes Simplex virus 1 and 2, Influenza A and B viruses, Parvovirus B19, and Hepatitis C Virus in HCV+ patients) or other microorganisms (e.g. Borrelia, Trypanosoma cruzi) If necessary, the molecular Laboratory should also be able to use paraffin-embedded biopsies Molecular tests can be performed in the Pathology Laboratory or sent to a Molecular Laboratory

A clinician's approach to myocarditis

Clinical presentation of myocarditis is extremely pleomorphic. Therefore, myocardial inflammation should always be considered in the presence of signs and symptoms that are not convincingly explained by alternative cardiac and non-cardiac causes. The ESC position statement clearly distinguishes between clinically suspected myocarditis and the effective diagnosis of myocarditis, so highlighting the need for histological confirmation as a gold standard. Clinically suspected myocarditis is defined by a clinical presentation consistent with the diagnosis and the presence of one or more abnormalities on non-invasive testing, particularly cMRI [4]. Unfortunately, the clinical, ECG, echocardiographic and laboratory abnormalities associated with myocardial inflammation and necrosis are largely non-specific. The ECG often shows repolarization abnormalities and QRS prolongation. Diffuse ST-T segment elevation without reciprocal changes is frequent in patients with an acute coronary syndrome like presentation, while atrioventricular conduction disturbances may suggest sarcoidosis or giant-cell myocarditis particularly when associated with ventricular arrhythmias. Common echocardiographic findings in patients with biopsy-proven myocarditis include global ventricular dysfunction and dilation, regional wall motion abnormalities and associated pericardial effusion, although isolated diastolic dysfunction has been also reported. Severe, diffuse systolic dysfunction in a non-dilated and thickened left ventricle can be detected in fulminant myocarditis due to hyperacute and extensive myocardial inflammation and oedema [5]. Laboratory tests including cardiac damage markers like Troponin I and T or neurohumoral markers like NT-proBNP indicate the presence of myocardial damage and dysfunction, respectively. In specific clinical settings, laboratory tests may be key in diagnosing or suspecting cardiac involvement associated with systemic diseases such as connective tissue disorders, vasculitis, sarcoidosis and hypereosinophilic syndromes [4].

cMRI is the most accurate and reliable non-invasive tool to detect myocardial inflammation. In recent years, the Lake Louise Criteria have been proposed to standardize an approach to myocarditis with the use of cMRI. These criteria are now included in the diagnostic algorithm proposed in the ESC consensus [150]. However, as previously discussed, these criteria suffer from major limitations. When compared to EMB in the setting of acute myocarditis, cMRI showed comparable sensitivity than EMB in patients with acute coronary syndrome-like presentation, but lower in patients with arrhythmic presentation or in the late, chronic heart failure stage [151]. In the near future, however, emerging tissue characterization techniques such as T1 and T2 mapping are expected to increase the accuracy of cMRI in detecting both acute and chronic, low-grade myocardial inflammation [152].

As discussed earlier, EMB remains the gold standard for the diagnosis of myocarditis [4]. According to the AHA/ACC/ESC guidelines, EMB is recommended in selected clinical scenarios including acute heart failure with haemodynamic or arrhythmic compromise (the latter due to AV block or ventricular arrhythmias), and chronic heart failure unresponsive to conventional treatment [153]. Conversely, a subsequent ESC statement proposed a diagnostic flow chart in which all patients with clinically suspected myocarditis should be referred to coronary angiography and EMB [4]. These divergent views are currently the object of considerable debate among cardiologists. Many see a systematic use of EMB as unjustified: although a safe procedure in expert hands, EMB remains an invasive procedure, and is indicated only when significant impact on treatment and prognostic stratification is expected (Fig. 8). As a particular case in point, EMB findings may be crucial when myocardial involvement is suspected in the context of a systemic inflammatory or autoimmune disease or when giant cell or eosinophilic or toxic myocarditis is suspected. In these scenarios, timely implementation of immunosuppressive treatment or withdrawal of the toxic agent may be life-saving [38, 76, 154].

Treatment of myocarditis

Management of patients with myocarditis is based on treatment of symptoms and aetiology-driven therapy.

Symptom management In acute myocarditis presenting with heart failure and in fulminant myocarditis, conventional pharmacological treatment may not suffice and supportive therapy including intra-aortic balloon pump, mechanical support by ventricular assist device and extracorporeal membrane oxygenation may be required. In patients with residual left ventricular dysfunction, long-term therapy with beta-blockers and ACE-inhibitors or ARBs is usually adopted. No specific recommendations for the treatment of arrhythmias in myocarditis are currently available and therapeutic approach follows standard guidelines for the management of ventricular arrhythmias

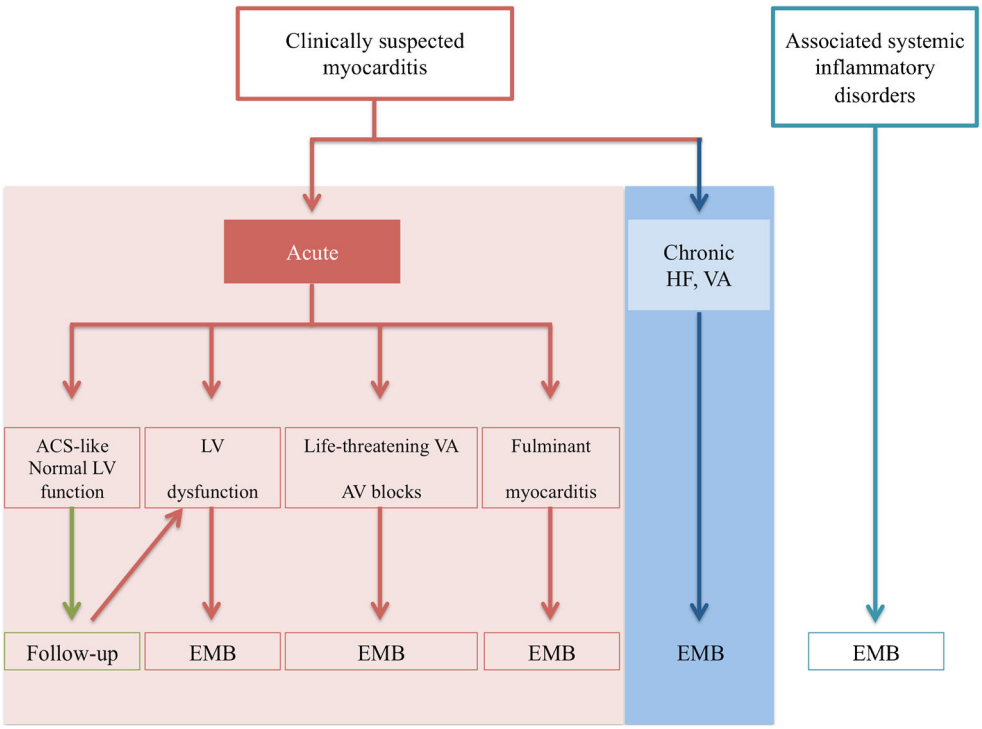

Fig. 8 Diagnostic flow chart showing cases of clinically suspected myocarditis requiring EMB
ACS acute coronary syndrome, *EMB* endomyocardial biopsy, *HF* heart failure, *LV* left ventricle, *VA* ventricular arrhythmia

and sudden death prevention. As myocarditis frequently heals completely, indications for implantable cardioverter defibrillator (ICD) implant, particularly in primary prevention, are controversial. In the absence of specific evidence, bridging by life-vest in patients presenting with severe ventricular arrhythmias is often adopted in order to evaluate recovery of cardiac function. An ICD is generally implanted at a later stage in the subset with residual dysfunction or arrhythmias. Ablation strategies have proven effective in reducing the arrhythmic burden, particularly when an epicardial approach is adopted [155]. Restriction of physical activity is recommended during the acute phase of myocarditis until the disease has completely resolved. Specifically, patients must be temporarily excluded from both competitive and leisure sports activity for at least 6 months, regardless of clinical presentation, treatment, age and gender.

Aetiology-driven therapy The ESC position statement recommends considering immunosuppression, in the absence of contraindications, in proven autoimmune (e.g. infection- negative) forms of myocarditis, including giant cell myocarditis, cardiac sarcoidosis and myocarditis associated with known extra-cardiac autoimmune disease. In the latter, immunosuppressive therapy is highly recommended [156]. Similarly, toxic and hypersensitivity myocarditis requires immediate withdrawal of the responsible agent and is generally responsive to high dose steroidal therapy. In acute viral or idiopathic myocarditis, the use of immunosuppression remains debated. The Myocarditis Treatment Trial showed negative results for immunosuppression but was limited in design, as it enrolled patients with myocarditis of unknown aetiology (i.e. without detailed pathologic information or assessment of viral genome in the myocardium), and different disease duration (acute and chronic myocarditis). Conversely, a single-centre controlled trial suggested a beneficial effect of combined steroid and azathioprine therapy in virus-negative chronic myocarditis [157], and similar data derives from a recent retrospective registry [158]. Based on available evidence, it seems reasonable to start immunosuppression in patients with clinical/pathologic chronic myocarditis; in these cases, ruling out active viral infection by EMB may be valuable. The most frequently adopted regimen of immunosuppression includes prednisone for 6 months (1 mg/kg/die for 1 month followed by 0.33 mg/kg/day for 5 months) and azathioprine (2 mg/kg/die) for 6 months on top of standard heart failure and antiarrhythmic therapy [157, 158]. The most frequently adopted regimen of immunosuppression includes prednisone (1 mg/kg/die for 1 month, then taper) and azathioprine (2 mg/kg/die for 6 months) on top of standard heart failure and antiarrhythmic therapy. Table 5 summarizes how a diagnosis of histopathologic subtypes of myocarditis by EMB may influence management.

Myocardial inflammation in inherited and acquired cardiomyopathies

Growing evidence shows that myocardial inflammation may play a role in the pathogenesis and/or clinical progression of several inherited and acquired cardiomyopathies. Imaging and pathology studies suggest that an inflammatory process is frequently involved, at least in a subset of patients and in specific stages, in determining the phenotype and the clinical manifestations of many cardiomyopathies. Myocardial inflammation has been frequently reported in patients with arrhythmogenic cardiomyopathy, caused by desmosomal protein gene mutations. Notably, the 'hot' phases of the disease characterized by enhanced ventricular arrhythmic propensity and progression of structural remodelling and fibrosis seem subtended by inflammatory relapses [165, 166]. Similarly, imaging and EMB studies have shown that myocardial inflammation is common in infiltrative and storage disorders including cardiac amyloidosis and Fabry disease cardiomyopathy, contributing to disease progression and influencing prognosis [167, 168]. More recently, inflammation has been proposed as a main determinant of structural and electroanatomical cardiac abnormalities in patients with Brugada syndrome, a disease sharing many features with arrhythmogenic cardiomyopathy [169]. Whether appropriate use of immunosuppressive strategies may help modify the clinical course of these genetic conditions is still unknown.

Conclusion

Diagnosing myocarditis is difficult both in terms of interpreting pathologic findings and putting them into a clinical context. An aetiology-targeted diagnostic work-up and close collaboration between clinicians and pathologists are essential for appropriate management. A viable model is the setting of heart transplantation, where collaboration between clinicians, pathologists and immunologists has, over time, allowed increasingly modulated and personalized therapies. There is an ongoing debate on important issues of rejection, i.e. alloantigen myocarditis: the significance of inflammatory burden; the relation between triggers, inflammation and myocardial injury and balancing cell and antibody-mediated immunity. All these issues require further investigation in the broader world of myocarditis, and integration of two different cultural perspectives: the practical action-oriented approach of the clinicians and the vocation of pathologists for a minute investigation of tissue in search of the cause of illness.

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Table 5 Impact of EMB histopathologic subtype diagnosis on aetiology-driven therapy

Histopathologic subtype of myocarditis	Most frequent aetiology	Histopathologic findings	Aetiology-driven therapy	Prognosis	Ref.
Lymphocytic myocarditis	Infectious: viruses	Inflammatory infiltrate: mainly lymphocytic; variable amount of neutrophils (related to myocyte damage) and macrophages (depending on stage) Extent of inflammation: focal, multifocal, diffuse; Myocyte damage/ necrosis: usually sparse or widespread; Fibrosis: depending on stage	Anti-viral drugs (beta interferon)[a] High-dose Ig[a]	Possible progression to chronic myocarditis/DCM if untreated	[157–161]
	Noninfectious: immune/autoimmune (post-viral immune; cardiac primary auto-immune; cardiac localization of immune systemic diseases)	As above with less evident myocyte damage and possible small vessel vasculitis	Immunosuppressive therapy (prednisone + azathioprine)	Full recovery with aetiology-driven therapy Possible progression to chronic myocarditis/DCM if untreated.	
Giant cell myocarditis	Autoimmune (primary or post-viral)	Inflammatory infiltrate: mixed with macrophages, lymphocytes, numerous scattered multinucleated giant cells, eosinophils and plasma cells Extent of inflammation: extensive Myocyte damage/necrosis: usually severe No well-formed granulomata Fibrosis: depending on stage	Combined immunosuppression (prednisone + azathioprine or/and cy-closporine and/or T cell depletion)	High mortality and recurrence rates Fulminant cardiogenic shock/malignant arrhythmias presentation	[162]
Sarcoidosis	Mixed genetic, environmental, infectious and immune	Inflammatory infiltrate: granulomatous with well-formed non-necrotizing epi-thelioid granulomas including giant cells, surrounded by T lymphocytes. Extent: multifocal/diffuse Myocyte damage/necrosis: usually limited Fibrosis: depending on stage	Steroids	Possibly complicated by AV blocks and ventricular arrhythmias	[163]
Eosinophilic myocarditis	Drug-related hypersensitivity reactions Churg Strauss syndrome Idiopathic hypereosinophilic syndrome Parasitic infections Malignancies	Inflammatory infiltrate: eosinophilic as major component or limited part of mixed inflammation (lymphocytes, macrophages, plasma cells, poorly formed microgranulomas and giant cells) Extent of inflammation: multifocal; diffuse Myocyte damage/necrosis: absent, focal/multifocal or atypically extensive Fibrosis: depending on stage	Inducing-drug withdrawal Steroids Monoclonal antibodies[a] Management of hypereosinophilia causes	Fulminant course frequent High rates of in-hospital death Full recovery possible if promptly treated	[76, 89, 164]
Other histopathologic patterns of drug-related myocarditis ('toxic myocarditis')	Drug/other substance-related direct toxic myocardial injury	Inflammatory infiltrate: paucicellular, mainly macrophages, occasional neutrophils and lymphocytes Extent of inflammation: sparse, multifocal Myocyte damage/necrosis microfoci of necrotic/damaged myocytes (hypereosinophilic, disrupted, fragmented, necrotic myocytes) and prominent contraction band necrosis. Fibrosis: usually absent or very focal	Inducing-drug/substance withdrawal Steroids	Full recovery if promptly treated. Possible progression to chronic myocarditis/DCM if untreated	[4, 164]

[a] Currently non-approved investigational therapies

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Abbreviations cMRI, Cardiac magnetic resonance imaging; ICD, Implantable cardioverter defibrillator; EMB, Endomyocardial biopsy; EGPA, Eosinophilic granulomatosis with polyangiitis; ESC, European Society of Cardiology; GCM, Giant cell myocarditis; HSM, Hypersensitivity myocarditis; HES, Idiopathic hypereosinophilic syndrome; IHC, Immunohistochemistry; LM, Lymphocytic myocarditis; PCR, Polymerase chain reaction; SCD, Sudden cardiac death; SLE, Systemic lupus erythematosus; TM, Toxic myocarditis

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