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Acute myocarditis in childhood: Linking early diagnostic indicators to aetiology and prognosis

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PhD THESIS

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Introduction

Acute myocarditis in childhood is a heterogeneous and potentially life-threatening condition, with clinical presentations ranging from mild chest pain to heart failure or fulminant forms. While often presumed to be a transient post-viral condition, growing evidence suggests that the condition may often involve additional contributing factors beyond infection alone. In particular, genetic susceptibility is increasingly recognised as a potential determinant of both disease onset and progression.

This thesis aims to characterise the aetiological spectrum, diagnostic challenges, and prognostic factors of paediatric myocarditis, with a particular focus on the role of early diagnostic indicators, including electrocardiogram, cardiac magnetic resonance imaging (CMR), endomyocardial biopsy (EMB), and genetic testing, in guiding clinical decision-making and improving outcomes.

Methods

This prospective multicentre study included paediatric patients (≤18 years) with myocarditis diagnosed according to the *European Society of Cardiology* and the *American Heart Association* over a 16-year period. The diagnostic protocol incorporated clinical assessment, electrocardiogram, echocardiography, blood biomarkers, CMR (applying 2018 Lake Louise criteria), EMB in selected cases, and next-generation sequencing genetic testing. Specific analyses were conducted in two subgroups: (1) infants presenting with new-onset heart failure and (2) adolescents with uncomplicated myocarditis. Follow-up evaluations, including CMR and clinical monitoring, extended for a minimum of 12 months. Data were analysed to identify early markers of aetiology and prognosis.

Results

In infants with new-onset heart failure, both EMB and CMR showed consistent evidence of myocardial inflammation. However, conventional diagnostic criteria for CMR and EMB were not sufficient to reliably differentiate between virus-mediated myocarditis and those carrying pathogenic genetic variants evolving towards cardiomyopathy. Genetic testing and microbiological tests were key for stratifying patients: those with pathogenic variants and

negative microbiological results had significantly worse outcomes, including persistent dysfunction and progression to cardiac transplantation.

Also in the infantile population, a specific ECG pattern, characterised by peaked P waves, low QRS voltages, and repolarization abnormalities, was identified in 70% of patients with Parvovirus B19 (PVB19) myocarditis, with a specificity of 98%, offering a highly accessible, non-invasive indicator of this viral aetiology.

In adolescents with infarct-like myocarditis, 22.2% were found to carry pathogenic or likely pathogenic variants in cardiomyopathy-associated genes, despite no family history of cardiac disease. Similarly to the infant cohort, among adolescents, those with genetic variants were not clinically or radiologically distinguishable at diagnosis. However, during follow-up, they exhibited higher recurrence rates and persistent myocardial abnormalities on follow-up CMR, including extensive late gadolinium enhancement and residual oedema.

Discussion and conclusion

This thesis provides novel evidence supporting the concept that some myocarditis may represent the first manifestation or "hot phase" of inherited cardiomyopathy. This redefines myocarditis not only as an acquired inflammatory condition but also as a potential early marker of an underlying genetic myocardial vulnerability.

This research highlights the diagnostic limitations of EMB and CMR in early stages, especially in infants, and demonstrates that the integration of genetic testing significantly improves diagnostic accuracy and risk stratification. On the other hand, the identification of a highly specific ECG pattern associated with PVB19 myocarditis offers a simple, cost-effective tool for early viral orientation, particularly valuable in settings with limited access to advanced diagnostics.

Furthermore, long-term follow-up was shown to be essential, even in apparently uncomplicated myocarditis, given the risk of a genetic background and the potential for late abnormalities to emerge over time in gene-positive patients. These findings advocate for a paradigm shift towards a personalised, multimodal diagnostic strategy integrating clinical, imaging, histological, microbiological, and genetic data to guide prognosis and therapy.

ABSTRACT IN CATALAN

Miocarditis aguda en la infància: Relació entre els indicadors diagnòstics precoços, l'etiologia i el pronòstic

Introducció:

La miocarditis aguda en la infància és una entitat clínica poc freqüent però potencialment greu, amb un ampli ventall de presentacions, des de dolor toràcic lleu fins a insuficiència cardíaca i formes fulminants. Tot i que tradicionalment s'ha considerat una malaltia transitòria post-viral, evidències recents apunten que poden intervenir-hi altres factors, especialment la susceptibilitat genètica, un potencial determinant tant de l'aparició com de la progressió de la malaltia.

Aquesta tesi té com a objectiu caracteritzar l'etiologia i identificar els principals reptes diagnòstics i factors pronòstics de la miocarditis pediàtrica; especialment la identificació precoç de marcadors diagnòstics mitjançant proves com l'electrocardiograma, la ressonància magnètica cardíaca (RMC), la biòpsia endomiocàrdica (BEM) i l'estudi genètic, per tal d'orientar la presa de decisions des del moment del diagnòstic.

Mètodes:

L'estudi es basa en una cohort prospectiva i multicèntrica de pacients pediàtrics diagnosticats de miocarditis segons els criteris de l'*European Society of Cardiology* i l'*American Heart Association* al llarg de 16 anys. El protocol diagnòstic va incloure l'avaluació clínica, electrocardiograma, ecocardiografia, biomarcadors, RMC (seguint els criteris de Lake Louise 2018), BEM en casos seleccionats i estudi genètic mitjançant *next generation sequencing*. Es van analitzar dos subgrups: lactants amb insuficiència cardíaca de nova aparició i adolescents amb miocarditis no complicada. El seguiment clínic i radiològic es va realitzar durant almenys 12 mesos.

Resultats:

En lactants amb insuficiència cardíaca de nova aparició, tant la BEM com la RMC van mostrar signes consistents d'inflamació miocàrdica. Tanmateix, els criteris diagnòstics convencionals de la RMC i la BEM no van ser suficients per diferenciar de manera fiable casos d'etiologia viral de casos amb presència de variants genètiques i evolució a miocardiopaties. L'estudi genètic i les proves microbiològiques van ser claus per estratificar els pacients: aquells amb

variants patogèniques i resultats microbiològics negatius van tenir una evolució clínica més desfavorable, incloent disfunció persistent i necessitat de trasplantament cardíac.

També en la població infantil, es va identificar un patró electrocardiogràfic específic (ones P picudes, baixos voltatges del QRS i anomalies de la repolarització), present en el 70% dels pacients amb miocarditis per Parvovirus B19 (PVB19), amb una especificitat del 98%, oferint una eina accessible i no invasiva per sospitar aquesta etiologia viral.

En adolescents amb miocarditis de tipus *infarct-like*, el 22,2% presentaven variants patogèniques o probablement patogèniques en gens associats a miocardiopaties, malgrat no tenir antecedents familiars coneguts de cardiopaties. Igual que en la cohort de lactants, en aquest subgrup, els pacients amb variants genètiques en gens associats a miocardiopaties no eren identificables ni des del punt clínic ni radiològic en el moment del diagnòstic. No obstant, durant el seguiment van mostrar taxes més elevades de recurrència i alteracions persistents en la RMC, com captació tardana de gadolini extensa i edema residual.

Discussió i conclusions:

L'evidència aportada dóna suport al concepte que la miocarditis pot ser la primera manifestació o "fase activa" d'una miocardiopatia hereditària, redefinint-la com un possible marcador precoç de vulnerabilitat genètica subjacent del miocardi.

Aquesta recerca destaca les limitacions diagnòstiques de la BEM i la RMC en les fases inicials i demostra que la incorporació sistemàtica de l'estudi genètic millora la precisió diagnòstica i l'estratificació del risc. D'altra banda, la identificació d'un patró electrocardiogràfic específic associat a la miocarditis per PVB19 permetria orientar precoçment l'etiologia viral, especialment útil en entorns amb recursos limitats.

Finalment, s'ha demostrat que el seguiment a llarg termini és essencial, fins i tot en casos aparentment no complicats, atesa la possibilitat d'una base genètica subjacent i del risc de progressió associat. Aquestes troballes recolzen un canvi de paradigma cap a una estratègia diagnòstica personalitzada i multimodal, integrant dades clíniques, d'imatge, histològiques, microbiològiques i genètiques per orientar el pronòstic.

ABBREVIATIONS

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DCM	Dilated cardiomyopathy
NDLVC	Non-dilated left ventricular cardiomyopathy
ARVC	Arrhythmogenic right ventricular cardiomyopathy
HCM	Hypertrophic cardiomyopathy
PVB19	Parvovirus B19
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
LVEF	Left ventricular ejection fraction
EMB	Endomyocardial biopsy
CMR	Cardiovascular Magnetic Resonance Imaging
ICU	Intensive care unit
ECMO	Extracorporeal membrane oxygenation
ICI	Immune checkpoint inhibitor
ECG	Electrocardiogram
PCR	Polymerase chain reaction
PET	Positron emission tomography
EGE	Early gadolinium enhancement
ECV	Extracellular volume
LGE	Late gadolinium enhancement
NGS	Next-generation sequencing
P/LP	Pathogenic or likely pathogenic
NSAID	Nonsteroidal anti-inflammatory drugs
ACEI	Angiotensin-converting enzyme inhibitors
MRA	Mineralocorticoid receptor antagonists
ARNI	Angiotensin receptor-neprilysin inhibitors
SGLT2I	Sodium-glucose cotransporter 2 inhibitors
VUS	Variant of unknown significance

INTRODUCTION

INTRODUCTION

I. OVERVIEW AND GENERAL CONCEPTS

Myocarditis is a pathological condition defined by an inflammatory injury to the myocardium, often accompanied by involvement of the cardiac conduction system and, in some cases, the pericardium [1-3]. This inflammation may arise from various aetiologies, principally infections, but also from exposure to toxic substances, or immune system activation, and represents a complex interplay between external or internal triggers and the host's immune response [4-5].

The disease is recognized as a potentially life-threatening condition due to its high mortality rates and the risk of significant complications, such as the progression to DCM. Despite its severity, myocarditis lacks a globally accepted treatment protocol, leading to significant variability in clinical management across institutions and geographic regions [6]. A further challenge lies in its frequent underdiagnosis during initial medical evaluations, as it may present with nonspecific symptoms in children, easily mistaken for benign viral or respiratory illnesses. Up to 83% of cases are initially misdiagnosed, delaying appropriate management [7,8].

Myocarditis represents a diagnostic and therapeutic challenge, primarily due to its highly heterogeneous clinical presentation, which ranges from mild and self-limiting cases to complicated myocarditis with acute heart failure, arrhythmias, or even sudden cardiac death [3,4]. This variability complicates its diagnosis and management, including the selection of appropriate diagnostic tests, determining the need for invasive procedures, optimizing the therapeutic interventions, and planning long-term follow-up strategies after recovery [9].

In the paediatric population, the current scientific evidence is limited. The low incidence of myocarditis, together with its clinical severity complicate the development of robust studies. Furthermore, no universally accepted diagnostic criteria exist for paediatric myocarditis, resulting in differences in diagnosis and management between centres [10]. Over the years, efforts have been made to standardize the diagnostic framework, but differences in interpretation and conceptual definitions of myocarditis within the scientific community have led to frequent revisions of its classification and criteria [1]. The most widely accepted definitions currently include the following:

Acute myocarditis:

Acute myocarditis is defined as an inflammatory disease of the myocardium diagnosed through histological, immunological, and immunohistochemical criteria [4]. Symptoms must have been present for less than one month to meet the criteria for acute presentation [1].

Inflammatory cardiomyopathy:

Inflammatory cardiomyopathy represents a broader diagnostic category that combines histological evidence (myocarditis) together with functional impairment. It is characterized by myocardial inflammation associated with systolic and/or diastolic dysfunction and ventricular remodelling [2,4,11]. This definition emphasizes the functional impact of inflammation on the affected myocardium. Importantly, inflammatory cardiomyopathy is not an exclusive diagnosis and may overlap with other conditions [4], such as dilated cardiomyopathy (DCM), non-dilated left ventricular cardiomyopathy (NDLVC) or arrhythmogenic right ventricular cardiomyopathy (ARVC) as defined in the 2023 guidelines on cardiomyopathies by the *European Society of Cardiology* [12].

Chronic inflammatory cardiomyopathy

Chronic inflammatory cardiomyopathy describes a persistent clinical syndrome characterized by ongoing myocardial inflammation and progressive ventricular remodelling lasting longer than one month. It often manifests as chronic heart failure, as a progressive disease without haemodynamic instability. Histological changes in this condition typically show signs of chronic inflammation, evidenced by less pronounced inflammatory infiltrates together with fibrosis, myocyte hypertrophy or necrosis [1,13].

II. EPIDEMIOLOGY

The true incidence of myocarditis is challenging to accurately determine due to discrepancies in diagnostic criteria [10] and the likelihood of underdiagnosis [7,14]. However, it is estimated that the incidence in the paediatric population is approximately 2 cases per 100,000 individuals per year [15]. While the incidence tends to increase with age, two distinct peaks of incidence have been identified (**figure 1**): the first occurring in children under the age of 2 and the second in adolescents [10,15,16]. A retrospective multicentre paediatric study found that 24% of patients were under 2 years old, while 46% were between 13 and 18 years old [16].

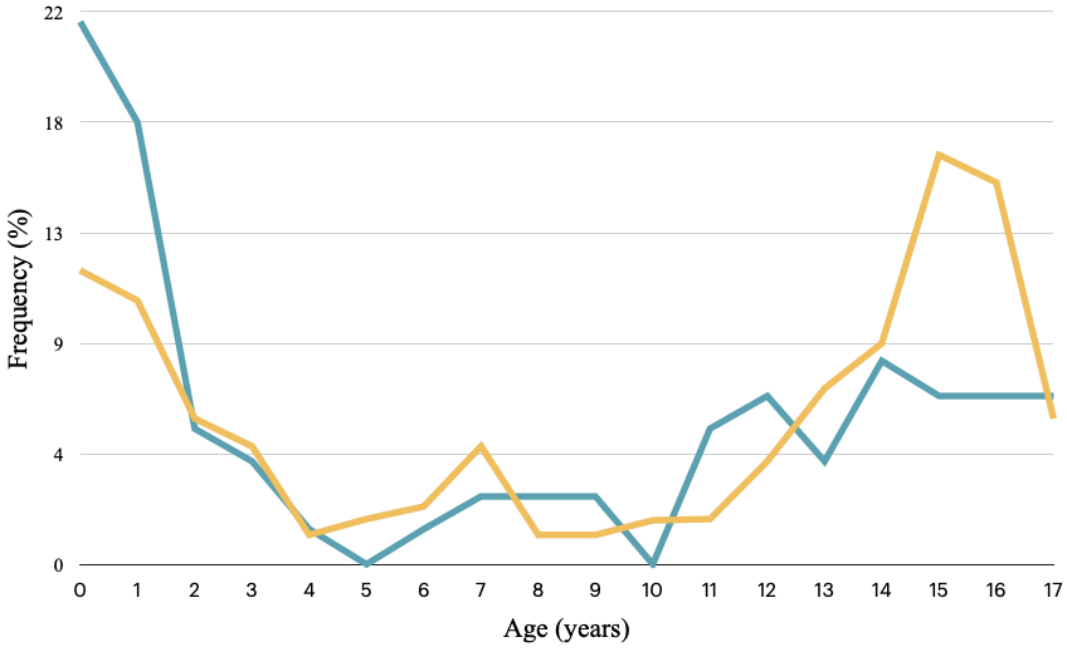


Figure 1. Age distribution histogram of paediatric myocarditis admissions in our 16-year cohort (blue line), compared with a 4-year international multicentre cohort from the United States (yellow line) [16].

The absolute prevalence and the relative proportion of the various aetiologies of myocarditis can fluctuate over time and across different geographic regions. These variations are influenced by factors such as widespread epidemics, endemic diseases, or the use of certain medications, which may act as triggers for the disease [1,2,17,18].

With regard to gender, the proportion of cases between males and females is generally equivalent up to the age of 6. However, beyond this age, the proportion of male cases increases significantly, reaching 64-77% of cases in adolescence [15,19]. Notably, despite having a similar clinical presentation at diagnosis, males tend to exhibit worse outcomes when compared to females. This gender disparity has been attributed to potential biological and immunological differences between the sexes, however, the exact mechanisms underlying these differences remain under investigation [20–22].

III. AETIOLOGY

Although the aetiology of myocarditis often remains unknown, a wide range of infectious agents, systemic diseases, medications, vaccines, and toxins can cause the condition (**table 1**)

[2,3,23]. Among these, infectious causes are the most prevalent, with viruses being the predominant agents [4,9].

Causes of myocarditis	
A. Infectious myocarditis	
Virus	**DNA viruses**: Parvovirus B19, herpesviruses (human herpesvirus 6, cytomegalovirus, herpes simplex virus, varicella-zoster virus), adenovirus, Epstein-Barr virus, smallpox virus, vaccinia virus. **RNA viruses**: Enteroviruses (coxsackievirus A and B), coronaviruses (SARS-CoV-2), influenza A and B, echovirus, poliovirus, respiratory syncytial virus, mumps virus, measles virus, rubella virus, hepatitis virus, dengue virus, yellow fever virus, Chikungunya virus, rabies virus, Lassa fever virus, Junín virus, human immunodeficiency virus (HIV).
Bacteria	*Staphylococcus, Streptococcus, Pneumococcus, Meningococcus, Gonococcus, Chlamydia, Clostridium difficile, Salmonella, Corynebacterium diphtheriae, Haemophilus influenzae, Mycobacterium tuberculosis, Mycoplasma pneumoniae, Francisella tularensis, Brucella, Borrelia burgdorferi* (Lyme disease) and *B. recurrentis, Treponema pallidum* (syphilis), *Leptospira* (Weil's disease), *Coxiella burnetii* (Q fever), *Rickettsia rickettsii* (Rocky Mountain spotted fever) and *R. tsutsugamushi.*
Parasites	*Trichinella spiralis, Taenia solium, Echinococcus granulosus.*
Fungi	*Actinomyces, Aspergillus, Blastomyces, Candida, Coccidioides, Histoplasma, Cryptococcus, mucormycosis, Strongyloides, Nocardia, Sporothrix.*
Protozoa	*Trypanosoma cruzi* (Chagas disease), *Toxoplasma gondii, Entamoeba, Leishmania, Balantidium, Sarcoystis, Plasmodium falciparum* (malaria).
B. Autoimmune myocarditis	
Autoimmune diseases or	Systemic lupus erythematosus, rheumatoid arthritis, eosinophilic granulomatosis with polyangiitis (Churg-Strauss syndrome), granulomatosis

immune disorders	with polyangiitis (Wegener's granulomatosis), Kawasaki disease, inflammatory bowel disease, scleroderma, polymyositis, myasthenia gravis, thyrotoxicosis, sarcoidosis, giant cell myocarditis, rheumatic fever.
Eosinophilic Myocarditis	**Hypersensitivity**: Drugs (beta-lactams, tetracyclines, fluoroquinolones, colchicine, furosemide, isoniazid, lidocaine, sulfonamides, phenytoin, phenylbutazone, methyldopa, thiazides, amitriptyline), tetanus toxoid, vaccines, serum sickness. **Hypereosinophilic syndrome.**
Mediated by autoantigens	Post-transplant myocarditis.
C. Toxic myocarditis	
Drugs	Cyclophosphamide, immune checkpoint inhibitors, anthracyclines, fluorouracil, lithium, catecholamines, hemin, interleukin-2, clozapine.
Substances	Amphetamines, cocaine, alcohol.
Heavy metals	Copper, iron, lead.
Hormones	Pheochromocytoma, vitamin B1 deficiency (beriberi).
Miscellaneous	Radiation, electrical discharge, scorpion stings, bee, wasp, or spider bites, snake bites, carbon monoxide, phosphorus, arsenic, sodium azide, hydrocarbon inhalation.

Table 1. Classification of myocarditis aetiologies [5,7,9].

The specific viruses implicated in myocarditis vary significantly depending on the geographic region studied and have also evolved over time. Historically, adenoviruses and enteroviruses were the most commonly identified pathogens. However, more recent findings have highlighted parvovirus B19 (PVB19) and human herpesvirus 6 as the primary viral agents associated with myocarditis. Additionally, the global severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has brought attention to myocarditis cases linked to this infection [2,18,24,25]. Nevertheless, the limited use of endomyocardial biopsy (EMB) and therefore the lack of microbiological studies on myocardial tissue represents a challenge in

accurately identifying the etiological distribution of myocarditis.

The most frequently implicated viruses in inflammatory cardiomyopathy can be categorized based on their tropism and persistence within the myocardium.

- **Cardiotropic viruses**, such as adenoviruses and enteroviruses (including coxsackie A, coxsackie B, and echoviruses), are capable of direct myocardial invasion but can typically be eliminated from the heart over time [26,27].

- **Vasculotropic viruses**, particularly PVB19 from the erythrovirus family, have the ability to persist within endothelial cells and may contribute to ongoing myocardial inflammation [24,28,29].

- **Lymphotropic viruses**, including members of the Herpesviridae family such as human herpesvirus 6, Epstein–Barr virus, and cytomegalovirus, can establish lifelong latency and have been detected in myocardial tissue, though their precise role in myocarditis remains debated [2].

- Some virus like human immunodeficiency virus, hepatitis C virus and influenza A and B viruses, do not directly infect cardiac tissue but can **induce myocarditis through immune activation** [30,31].

- Coronaviruses, including SARS-CoV-2, have gained attention due to their use of **angiotensin-converting enzyme 2 receptors**, which are widely expressed in cardiomyocytes, endothelial cells, and pericytes. While these coronaviruses have been associated with cardiac inflammation, the extent to which they directly contribute to myocardial injury versus triggering systemic inflammatory responses remains an area of active research [2,18].

The mechanisms by which these viruses contribute to the development of myocarditis are explored in the following section.

IV. PATHOFISIOLOGY

The pathophysiology of myocarditis varies significantly depending on the specific underlying cause [9]. A schema of its pathophysiology is shown in **figure 2**.

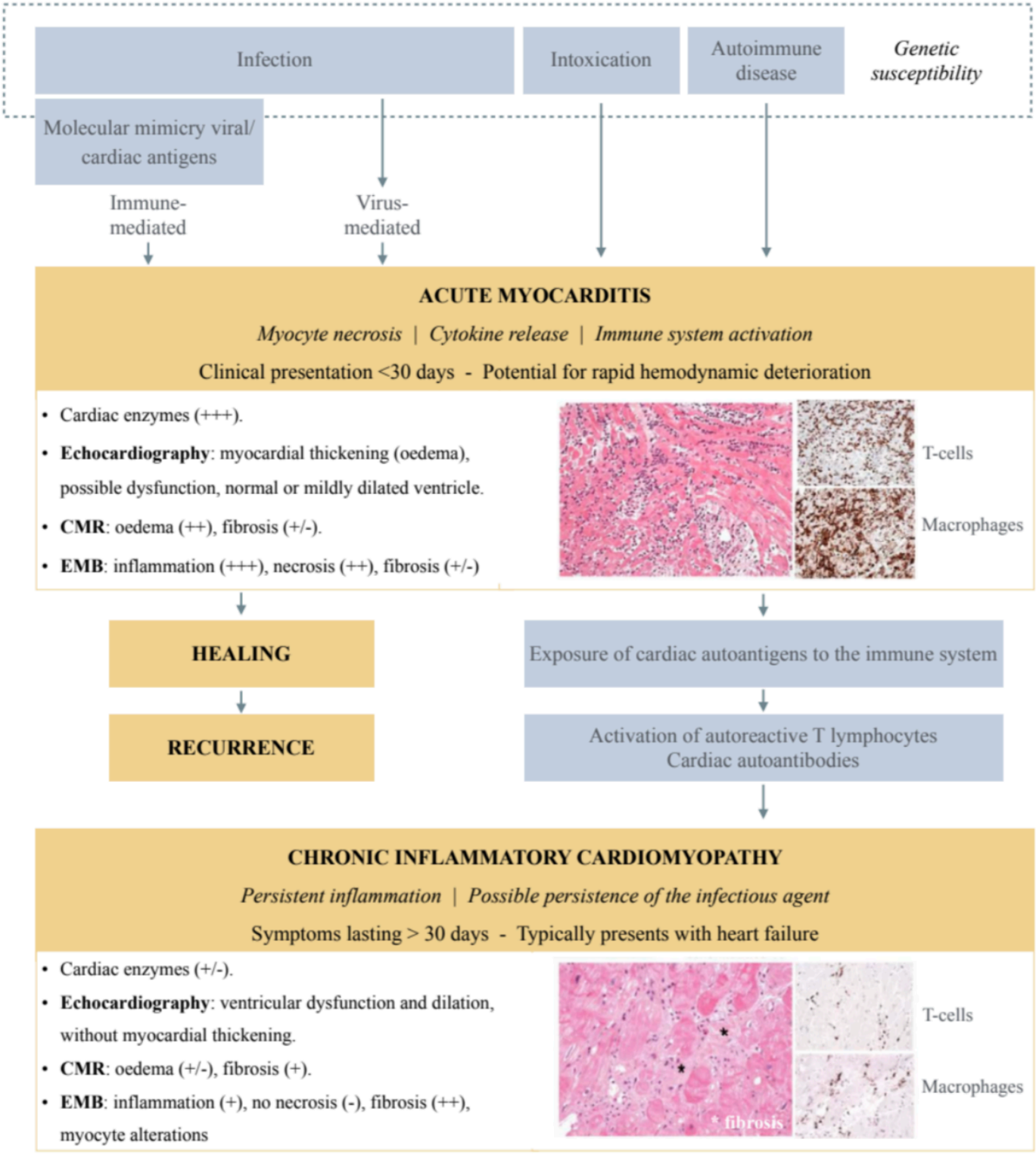


Figure 2. Pathophysiology of myocarditis [1,7]

Among the diverse aetiologies, viral myocarditis is the most frequent, and within this category, it is essential to distinguish between two primary mechanisms of myocardial injury:

- **Virus-mediated myocarditis:** This mechanism occurs when direct viral cytotoxicity specifically targets the myocardium. To date, direct myocardial damage has been confirmed only for a few viruses, such as enteroviruses, particularly coxsackieviruses, and adenoviruses [1,11]. The potential for direct cytotoxicity by other viruses remains uncertain.

- **Immune-mediated myocarditis**: This form of myocarditis occurs as a result of an immune response triggered by viral infections, even in the absence of viral genome in the myocardial tissue. Examples of this mechanism include respiratory viruses as influenza or coronaviruses, where the response of the host's immune system to the infection leads to inflammation and injury to the myocardium, rather than direct viral invasion [2,30,32].

Except for enteroviruses (such as coxsackievirus), which have been shown to cause direct myocardial damage [26,31], particularly in neonates [27], current evidence suggests that **immune-mediated** reactions are the predominant mechanism of myocardial injury for most other viruses (human immunodeficiency virus, hepatitis C virus, influenza A and B viruses). Although there remains some controversy, it is hypothesized that the causal mechanism involves molecular mimicry between viral and cardiac antigens. This mimicry leads to the infiltration of autoreactive T lymphocytes into the myocardium, creating and perpetuating inflammation and contributing to pathological myocardial remodelling [33,34]. PVB19, the most common virus implicated in myocarditis, may induce both virus-mediated and immune-mediated myocarditis, particularly in children [28,29,35].

The pathophysiological mechanisms of myocarditis have been extensively studied using animal models, with coxsackievirus myocarditis being the most thoroughly studied prototype. Coxsackievirus enters myocardial cells by utilizing specific receptor CAR (coxsackievirus and adenovirus receptor)**,** which is highly expressed in cardiac tissue [36]. During the first two weeks of infection, the virus causes myocyte necrosis through viral replication, representing a **virus-mediated** mechanism [26,37]. Following this initial phase, the viral damage to the myocardium triggers a pathological inflammatory response involving both the innate and adaptive immune systems, representing the immune-mediated component of myocardial injury. The innate immune system is activated via Toll-like receptors (TLRs), particularly TLR-3 and TLR-4, which recognize pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs). This activation leads to the release of inflammatory mediators

such as tumour necrosis factor-alpha (TNFα)**,** interleukin-1 beta (IL-1β)**,** interleukin-6 (IL-6), and nitric oxide. These mediators recruit immune cells residing in the heart, including natural killer cells and macrophages, and stimulate the release of type I interferons in an attempt to eliminate the virus. The inflammatory mediators also promote fibroblast activation, resulting in the production of cytokines and chemokines, which, in turn, stimulate the bone marrow to produce neutrophils and monocytes. Monocytes are among the principal infiltrating cells in the heart during myocarditis. This innate immune response subsequently triggers the adaptive immune system, leading to the proliferation of antigen-specific T and B lymphocytes. T lymphocytes can target viral proteins and, through molecular mimicry during myocarditis, attack the host's cardiac tissue. The release of cardiac myosin from damaged cardiomyocytes further amplifies the inflammatory response by acting as a ligand for Toll-like receptors [2,11,26,38]. B cell activation and the production of cardiac-specific autoantibodies, such as those targeting myosin or β1-adrenergic receptors, contribute to sustained myocardial inflammation, especially if viral replication persists, as it is associated with a worse prognosis and a higher risk of progression to chronic myocardial dysfunction [26,39-43].

While the pathophysiology of PVB19 myocarditis is less well-characterized than that of coxsackievirus, existing data suggest a distinct mechanism. PVB19 can induce **both virus-mediated and virus-triggered myocarditis** [24,28,35]. The virus infects endothelial cells of intramyocardial blood vessels via the erythrocyte P antigen as its primary receptor, using integrin αvβ1 as a coreceptor [44]. Once inside the endothelial cells, PVB19 triggers the release of pro-inflammatory cytokines. PVB19 genome encodes two major proteins, NS1 (a non-structural protein) and VP1/2 (the capsid protein), along with two smaller accessory proteins whose functions remain largely unknown. The NS1 protein plays a crucial role in viral replication, cytotoxicity, and host-cell apoptosis. Additionally, the phospholipase activity of VP1/2 is believed to contribute to the development of autoimmunity. Studies have shown that immunization with VP1/2 can induce DCM in murine models [45]. Sustained inflammation and endothelial dysfunction, particularly during the phase of viral persistence, can eventually lead to myocyte necrosis [24,35].

Viruses belonging to the Herpesviridae family, including human herpesvirus 6, Epstein–Barr virus, and cytomegalovirus, have also been linked to myocarditis. Unlike enteroviruses, which cause direct myocardial damage, herpesviruses primarily affect immune cells and may indirectly lead to myocardial inflammation. Human herpesvirus 6, in particular, can integrate

into the human genome and persist lifelong in a latent state and is still unknown if its reactivation plays a causative role in myocarditis or is merely an epiphenomenon [2, 46].

V. GENETIC INSIGHTS INTO MYOCARDITIS

Genetic susceptibility to myocarditis

Although myocarditis has traditionally been considered an acquired condition triggered by infectious, autoimmune, or toxic factors, recent research has highlighted the role of genetic predisposition in influencing both susceptibility and disease progression [47-51]. The fact that life-threatening myocarditis is rare in children despite the widespread circulation of causative viruses suggests that underlying genetic factors may determine individual vulnerability to myocardial injury [52,53].

Genetic variants associated with cardiomyopathies may predispose the myocardium to acute inflammatory injury following viral infection, immune dysregulation, or other environmental stressors [54,55]. This vulnerability may contribute to myocardial infection by pathogens, altered immune responses, and impaired myocardial repair mechanisms. However, investigating genetic associations in myocarditis remains complex due to the extreme heterogeneity in clinical presentation, diagnostic methods, and patient cohorts across different studies [48].

In myocarditis, the combination of myocardial oedema, fibrosis, endothelial dysfunction, electrical abnormalities, and activation of inflammatory cascades can contribute to ventricular dysfunction, particularly in genetically predisposed individuals [51]. Genetic background should always be considered, and family history should be analysed when trying to identify high-risk patients, as those with a family history of myocarditis have a higher likelihood of carrying pathogenic variants and worse outcomes [56].

Pathogenic variants in myocarditis

Recent genetic studies have revealed that between 8% and 22% of patients initially diagnosed with myocarditis harbour pathogenic or likely pathogenic (P/LP) variants in cardiomyopathy-associated genes [51,53,56,57]. These genetic variants are particularly overrepresented in patients with myocarditis presenting with heart failure or arrhythmic phenotypes, whereas patients with uncomplicated myocarditis are less likely to carry these variants [57,58].

A recent meta-analysis including both paediatric and adult cohorts estimated an overall prevalence of cardiomyopathy-associated variants in myocarditis patients at 14.5%. The prevalence was only 4.2% in patients with uncomplicated myocarditis but significantly higher in complicated cases, with 21.9% in adults and up to 45% in children presenting with acute heart failure or life-threatening arrhythmias [47]. The most commonly identified genetic variants in myocarditis involve genes encoding sarcomeric (51%) and desmosomal (17%) proteins, while others are linked to sarcolemma proteins (7%), cochaperone heat shock proteins (7%), ion channels (1%), RNA-binding proteins (4%), DNA-binding proteins (1%), cytoskeletal proteins (5%), nuclear envelope components (6%), and the sarcoplasmic reticulum (3%) [47].

In patients with infarct-like myocarditis and preserved left ventricular function, truncating variants in *DSP* have been most frequently reported, along with variants in *DSG2* and *PKP2* [49-51]. Although further research is needed, *DSP* appears to be the most frequently found desmosomal gene implicated in myocarditis, as it encodes desmoplakin, a major intracellular component of the desmosome, which plays a crucial role in maintaining intercellular adhesion [54]. Notably, 15% of adults diagnosed with cardiomyopathy and known *DSP* variants had experienced episodes of myocardial injury resembling myocarditis, reinforcing the overlap between myocarditis and desmosomal cardiomyopathies [60].

Conversely, in patients with reduced left ventricular function, genetic variants in sarcomeric genes, particularly *TTN* truncating variants, followed by *TNNT2, MYH7, TNNI3, TNNC1, MYBPC3*, and *TNNT1*, are more prevalent [47,51]. This suggests that P/LP variants in cardiomyopathy genes may underlie paediatric myocarditis characterized by early-onset heart failure, likely by compromising myocardial structural integrity and function [57].

The second hit hypothesis

An emerging concept in myocarditis pathogenesis is the "second hit" hypothesis, in which an initial genetic predisposition increases susceptibility to myocardial inflammation triggered by an infectious, autoimmune, or environmental factor. Underlying genetic variants affecting cardiomyocyte structural proteins may weaken myocardial integrity, making the heart more vulnerable to viral infection, immune-mediated injury, or inflammatory stressors [48,52,54].

A similar pattern is seen with *TTN* truncating mutations, which have been implicated in alcoholic cardiomyopathy, peripartum cardiomyopathy, and chemotherapy-related

cardiomyopathy, suggesting that a second hit from environmental factors, in addition to genetic predisposition, leads to cardiomyopathic remodelling [61]. A similar mechanism has been proposed in arrhythmogenic cardiomyopathies, where additional genetic variants, strenuous exercise, or other physiological stressors accelerate disease onset and progression [54].

Although the mechanistic link between cardiomyocyte death and inflammation remains unclear, studies suggest that genetic abnormalities may prime the myocardium for inflammation, necrosis, and subsequent fibrotic remodelling. Further research is needed to clarify whether inflammatory triggers initiate myocardial damage in genetically susceptible patients or whether inflammation is a reactive phenomenon following myocyte loss [51,62].

Myocarditis as an early phenotype of cardiomyopathy (hot phases)

Acute myocarditis can be the first clinical manifestation of DCM, NDLVC, or ARVC [47,51]. Episodes of chest pain, ECG abnormalities, troponin elevation, and CMR evidence of myocardial inflammation have been described as "hot phases", which may represent an early sign of disease progression [62-64].

These “hot phases” may recur over time, with some patients experiencing multiple myocarditis episodes [63]. Although recurrent myocarditis remains rare, it has been recognized as a risk factor for an underlying P/LP variant [65]. In a recent study, more than one-third of patients with recurrent acute myocarditis were later diagnosed with NDLVC or ARVC, reinforcing the idea that early myocarditis episodes may represent an inflammatory stage of genetic cardiomyopathies [66]. Given this overlap, myocarditis should be evaluated alongside genetic testing and family history assessment, as this could help identify patients at risk of progressive cardiomyopathy [50,67].

Overlap with cardiomyopathies

Acute myocardial inflammation, detected by either EMB or CMR, has been observed in patients with DCM, NDLVC, and ARVC, complicating the differentiation between myocarditis and inherited cardiomyopathies [62,63,68]. Myocardial oedema, fibrosis, endothelial dysfunction, and immune activation may all contribute to ventricular dysfunction in genetically predisposed individuals [67]. Notably, the presence of myocardial inflammation has been associated with worse clinical outcomes in cardiomyopathies [64].

In cases of severe left ventricular dysfunction, distinguishing between complicated myocarditis and primary cardiomyopathy at initial presentation is often challenging [69]. Moreover, heart failure itself is known to trigger myocardial inflammation, making the identification of inflammation via EMB or CMR insufficient to confirm a diagnosis of myocarditis [70].

Recent studies suggest that inflammation has a significant role in arrhythmogenic cardiomyopathies [63,66]. Myocardial inflammation has been detected in these patients, with minimal inflammatory foci described as an early sign of disease progression [68,72]. Specifically, in patients carrying *DSP* variants, myocardial inflammation can appear even in the absence of infectious or external triggers, a phenomenon referred to as "sterile" inflammation [73]. The underlying mechanism remains unclear, but it is hypothesized that desmosomal disruption compromises intercellular adhesion, leading to myocardial injury and an inflammatory response [64]. Additionally, reduced expression of desmosomal genes has been shown to activate inflammatory pathways, which may exacerbate myocardial inflammation in the presence of additional stressors, such as strenuous exercise [54]. This inflammatory process contributes to impaired contractility and an increased risk of ventricular arrhythmias.

VI. CLINICAL PRESENTATION

The clinical presentation of myocarditis is highly variable, ranging from asymptomatic or subclinical forms to life-threatening conditions such as cardiogenic shock [1,4,9]. Furthermore, myocarditis is a relatively frequent cause of sudden cardiac death in young individuals, accounting for 6–10% of cases in autopsy-based series [74,75]. The signs and symptoms of myocarditis are often heterogeneous and nonspecific, which can result in delayed or missed diagnosis [7,8,26]. Studies estimate that 41% to 69% of paediatric cases of acute myocarditis are preceded by viral prodromes and frequently by fever [9,76].

Most common symptoms and signs in the paediatric population are shown in **table 2**.

Symptoms	Signs	Associated events
Fatigue (25-70%)	Tachypnoea (52-60%)	Viral prodromes (41-69%)
Shortness of breath (35-69%)	Tachycardia (32-57%)	Arrhythmias (11-45%)
Fever (31-58%)	Hepatomegaly(21-50%)	Syncope (4-10%)

Nausea, vomiting, abdominal pain (28-48%) Rhinorrhoea (38-44%) Chest pain (24-42%) Dyspnoea (22-25%) Cough (17-44%) Palpitations (16%) Diarrhoea (8%)	Respiratory distress (21-47%) Heart murmur (26%) Gallop rhythm (20%) Weak pulses (16-21%) Oedema (7%) Cyanosis (2%)	Sudden cardiac death (unknown in paediatrics)

Table 2. Common symptoms and signs of myocarditis in the paediatric population (*Adapted from: N Engl J Med. 2009;360(15):1526–38*) [5].

Clinically, patients can be classified into two major categories: **uncomplicated myocarditis** and **complicated myocarditis**, the latter encompassing those clinical presentations with ventricular dysfunction, arrhythmias, or cardiogenic shock [1,4,77]. Compared to adults, children, and particularly infants, tend to present with more severe forms of the disease, with complicated myocarditis reported in 38% of paediatric cases [10]. Based on clinical and diagnostic features, four major subgroups of myocarditis can be identified:

Uncomplicated myocarditis or infarct-like pattern:

This is characterized by chest pain associated with electrocardiographic abnormalities, often including ST-segment elevation, and elevated troponin levels, and without significant left ventricular dysfunction [78,79]. This presentation mimics acute coronary syndromes. It represents the majority of myocarditis cases, typically affects individuals over the age of 10 years and is generally associated with a more favourable prognosis [3,6,52].

Complicated myocarditis with a cardiomyopathic pattern:

This form involves significant systolic dysfunction, leading to clinical manifestations of heart failure. Patients often present with fatigue, dyspnea, hepatomegaly, and other signs of volume overload. This subgroup is associated with worse clinical outcomes [3,77].

Complicated Myocarditis with Arrhythmias

This includes both ventricular and supraventricular tachycardias, as well as atrioventricular blocks although these are a rare presentation [80-82]. Arrhythmias can significantly complicate the clinical course, requiring prompt recognition and management to mitigate the risk of sudden cardiac arrest or other life-threatening complications. Arrhythmias have been reported in up to 45% of young patients during hospitalization [82] and may recur after discharge [80].

Several pathophysiological mechanisms have been proposed to explain the presence of arrhythmias in myocarditis, including [2]:

- Electrical instability caused by direct cytopathic effects of the virus.
- Coronary microvascular or macrovascular ischemia.
- Gap junction dysfunction leading to impaired intercellular electrical coupling.
- Alterations in calcium currents.
- Involvement of the cardiac conduction system.

Fulminant myocarditis

This form is characterized by haemodynamic instability resulting from cardiogenic shock, necessitating the use of inotropes or mechanical circulatory support such as extracorporeal membrane oxygenation (ECMO) to maintain adequate perfusion and prevent multi-organ failure [83-85]. Fulminant myocarditis is more frequently observed in paediatric populations compared to adults and is associated with a high risk of mortality or the need for heart transplantation [84,86].

VII. DIAGNOSTIC APPROACH

Although there is ongoing debate among experts regarding whether myocarditis should be primarily defined by histological criteria or by a predominantly clinical approach, in routine clinical practice, the diagnosis is often based on clinical presentation together with elevated biomarkers of myocardial necrosis, and the non-invasive identification of myocardial inflammation using CMR [3,16,19,87]. Various diagnostic approaches have been proposed for

myocarditis, with different scientific societies establishing distinct classification systems in their clinical guidelines.

The *American Heart Association*, in its 2021 paediatric clinical guidelines [9], proposes a four-tiered diagnostic framework for myocarditis:

1. **Possible myocarditis**: Based on suggestive symptoms without confirmatory findings.
2. **Clinically suspected myocarditis**: Characterized by symptoms and supportive laboratory features.
3. **CMR-confirmed clinically suspected myocarditis**: Where cardiac magnetic resonance identifies features consistent with myocardial inflammation. Currently, biopsy rates are declining as practitioners increasingly rely on CMR.
4. **Biopsy-proven myocarditis**: Where EMB provides definitive histopathological confirmation. Although positive histological results prove myocarditis, it is important to note that a negative EMB or CMR result does not necessarily exclude the diagnosis of myocarditis, given the focal nature of myocardial inflammation and the potential limitations of sampling techniques [9].

In contrast, the European approach to myocarditis diagnosis is guided by the 2013 *European Society of Cardiology (ESC)* position statement [4]. This framework is primarily focused on adult patients and categorizes cases into two key groups:

1. **Clinically suspected myocarditis**: The clinical suspicion of myocarditis, as per the ESC guidelines, is established when patients present with compatible clinical symptoms (e.g., chest pain, dyspnoea, palpitations) and supportive findings from diagnostic tests, including elevated troponins, electrocardiographic changes, echocardiographic abnormalities, or characteristic CMR findings.
2. **Histologically confirmed myocarditis**: Requiring EMB for definitive diagnosis, after excluding coronary artery disease and other structural cardiac disorders that could account for the patient's clinical presentation.

Finally, the *American College of Cardiology* introduced a new four-stage classification system for myocarditis in late 2024, aiming to better define the spectrum of the disease [78]. They acknowledge that some individuals are at risk of developing myocarditis but remain disease-

free, while others exhibit subclinical myocardial injury or inflammation without overt symptoms. The proposed classification aligns with the established heart failure staging model described in the 2022 American management guidelines [88], which outlines disease progression from risk factors to advanced stages.

1. **Stage A**: This stage includes **individuals who are at risk** of developing myocarditis but do not show signs of myocardial injury or inflammation. Risk factors include genetic predisposition, a personal history of myocarditis, immune checkpoint inhibitor (ICI) therapy, autoimmune disorders, or exposure to cardiotoxic substances.

2. **Stage B**: Refers to **asymptomatic individuals** who exhibit objective signs of myocardial inflammation or injury, such as inflammation on CMR or elevated troponin levels, provided these biomarkers are accompanied by additional supportive evidence of myocarditis. Patients in this stage do not have any clinical symptoms by definition, raising questions regarding the best approach for identifying and monitoring them.

3. **Stage C**: Represents **symptomatic myocarditis**, where patients develop clinical manifestations such as chest pain, dyspnoea, palpitations, arrhythmias, or heart failure symptoms. This stage correlates with active myocardial inflammation.

4. **Stage D**: Defines **advanced myocarditis**, characterized by severe haemodynamic instability requiring inotropic support, vasopressors, or temporary mechanical circulatory assistance like ECMO. It also includes patients experiencing life-threatening electrical instability, such as ventricular arrhythmias or conduction disturbances requiring urgent intervention.

These differing diagnostic frameworks reflect the evolving nature of myocarditis classification and the challenges in achieving a universally accepted definition. While histological confirmation remains the gold standard, practical limitations, including the invasive nature of EMB, imply that many cases are diagnosed based on clinical and imaging criteria.

VIII. COMPLEMENTARY INVESTIGATIONS

Chest X-ray

Chest X-ray is commonly performed in patients with suspected myocarditis, mainly to assess

cardiac size and pulmonary involvement. The most common finding on chest X-ray in myocarditis is cardiomegaly, reflecting ventricular dilation or myocardial oedema [6,7].

Electrocardiogram (ECG)

The ECG is a widely used initial screening tool for myocarditis diagnosis, despite its low sensitivity of approximately 47% [5]. ECG abnormalities are observed in 85–96% of myocarditis cases and in nearly all affected paediatric patients [89]. However, ECG findings are nonspecific, making it difficult to establish a definitive diagnosis based solely on electrocardiographic patterns [77,90,91]. While in adults, myocarditis is often associated with concave, widespread ST-segment elevation [4,25,77], in paediatric patients, other ECG patterns are more commonly reported, including T-wave inversion and low voltage QRS complexes [9,24,92].

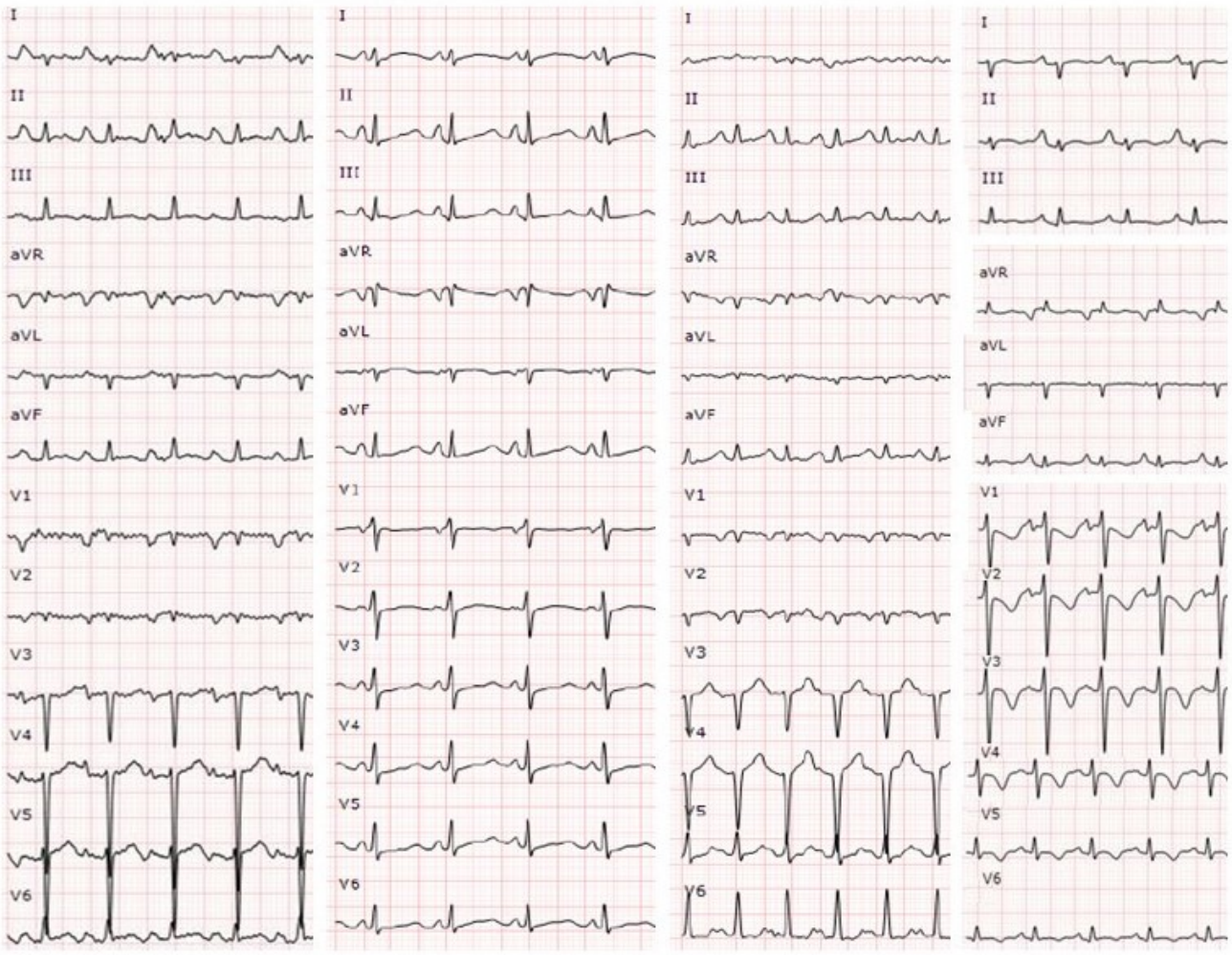


Figure 3. Representative ECG examples demonstrating peaked P waves, low QRS voltages, and repolarization abnormalities (manifested as negative or flattened T waves with or without QTc prolongation) in the limb leads.

Certain ECG abnormalities are associated with a poorer prognosis: pathological Q waves, wide QRS complexes, a QRS/T angle of 100° or greater, prolonged QT intervals, high-degree atrioventricular block, and malignant ventricular tachyarrhythmias. Conversely, ST-segment elevation with a pattern of early repolarization has been associated with better prognosis [90-93].

Specific ECG alterations in myocarditis:

- **PR segment depression**: More frequently observed in myopericarditis, as pericardial inflammation can disrupt epicardial action potentials, leading to atrial repolarization irregularities. Similar findings may occur in isolated myocarditis with primary atrial involvement [90,92].

- **Low QRS voltage**: Often linked to pericardial effusion due to the increased electrical resistance from accumulated fluid. However, 18% of acute myocarditis patients demonstrate low QRS voltage independent of pericardial effusion, possibly due to ventricular wall oedema, pulmonary congestion, or peripheral oedema [5,94].

- **Pathological Q waves**: Typically observed in the inferior and lateral leads, these are more frequent in infarct-like myocarditis and fulminant myocarditis, though their prevalence in myocarditis is generally below 20% [90,91]. Early resolution suggests reversible myocardial injury and resolution of inflammation, whereas persistent Q waves are associated with worse outcomes [90,94].

- **Wide QRS complexes**: Reflect structural damage to cardiomyocytes or the cardiac conduction system. With a variable prevalence between studies, wide QRS complexes correlate with disease severity, poor prognosis, and early identification of severe disease progression [90-93].

- **ST-Segment elevation**: Resulting from subepicardial myocardial injury, this abnormality is more prominent in myopericarditis and is typically transient, resolving in 24–48 hours [92,93].

- **T-Wave inversion**: The most common abnormality in paediatric acute myocarditis, reported in 9–48% of cases, and observed in both complicated and uncomplicated forms of the disease. It is generally considered a late ECG finding [90-93]. T-wave inversion is believed to result from regional or transmural repolarization inhomogeneity due to acute myocardial inflammation or oedema. It strongly correlates with T2-weighted CMR findings but not with contrast enhanced CMR patterns, suggesting it reflects acute inflammation rather than irreversible myocardial damage. Since myocardial oedema is transient, T-wave inversion lacks prognostic significance [95].

- **QT interval prolongation:** Though it is not very prevalent, QT prolongation is a clinically significant finding, as it is a known arrhythmogenic trigger and is associated with poor clinical outcomes in myocarditis [90].

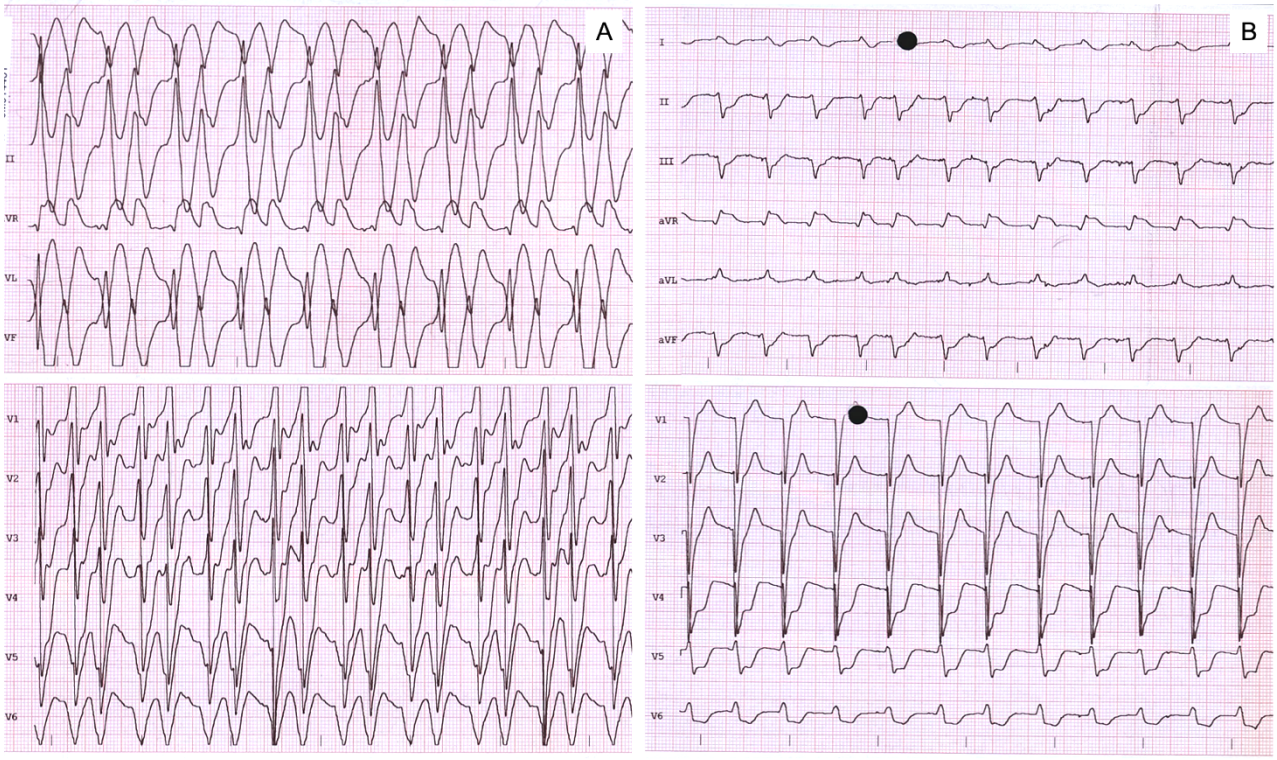


Figure 4. ECG from a patient with diphtheritic myocarditis. The initial ECG (A) shows a regularly irregular ventricular tachycardia with two alternating cycle lengths. Following antiarrhythmic treatment with amiodarone (B), the ECG demonstrates an irregular ventricular rhythm with capture beats.

Laboratory biomarkers

Although laboratory biomarkers can help detect myocardial injury and aid in diagnosing myocarditis, they lack specificity for distinguishing myocarditis from other causes of myocardial dysfunction [6,9,96]. Many biomarker categories have been studied in this context:

- **Inflammatory biomarkers (C-reactive protein, erythrocyte sedimentation rate, leukocyte count)**: Widely available and easily obtainable, however, while they may be elevated in myocarditis, their increase is nonspecific and can result from any inflammatory process, making them neither sensitive nor specific for myocarditis diagnosis [4,9,96].

- **Myocardial necrosis markers (troponins, creatine kinase)**: These markers provide evidence of cardiac injury. Troponins are elevated in 64–100% of myocarditis patients and are highly sensitive indicators of myocardial damage [3,6]. Troponin is more sensitive and specific than creatine kinase for diagnosing myocarditis. In most patients with myocarditis, troponin levels decline within 24 hours, particularly in those with favourable outcomes [97,98]. Elevated troponin levels correlate with a higher risk of mortality or the need for mechanical circulatory support, although they do not necessarily predict ventricular dysfunction or arrhythmia development [16,82,99].

- **N-terminal pro-B-type Natriuretic Peptide (NT-proBNP)**: It is released in response to ventricular stretch and pressure overload [97,100]. BNP, its active product, binds to natriuretic peptide receptors to activate guanylate cyclase and increases levels of cyclic guanosine monophosphate to induce vasodilation, natriuresis, and diuresis. Additionally, it reduces renin-angiotensin-aldosterone system activity, decreases ventricular stiffness, and improves lusitropy [96,100]. NT-proBNP levels correlate with ventricular dysfunction and symptom severity and are associated with poorer prognosis in myocarditis [96,97,100].

- **Emerging biomarkers** have been proposed for diagnosing and monitoring myocarditis. However, they remain experimental and are not yet part of routine clinical practice. Some of the most promising include:

    - **MicroRNAs and circulating cell-free DNA**: These non-coding RNA fragments and genetic materials released from damaged cells serve as potential indicators of

myocardial inflammation and cell death, reflecting mechanisms such as apoptosis, necrosis, and autophagy [101].

- **Soluble ST2 receptors**: Markers of inflammation and myocardial stress, they provide independent prognostic value, unaffected by renal function, making it a reliable indicator of ventricular dysfunction and disease progression [96,101].
- **Galectin-3**: A soluble peptide secreted by activated macrophages, involved in collagen secretion and fibrosis. It functions as a marker of myocardial remodelling, particularly relevant in paediatric heart failure involving fibrotic progression [101].
- **Cardiac autoantibodies**: These antibodies target structural, sarcoplasmic, or sarcolemmal proteins of the myocardium, revealing the immune-mediated mechanisms of myocarditis. Their presence has been linked to progression to DCM and could potentially be used for screening family members at risk of developing myocardial dysfunction [1,4,96].
- **Other novel biomarkers**, including MR-proANP, MR-proADM, GDF-15, and matrix remodelling markers (e.g., MMPs and TIMPs), are under investigation for their role in fibrosis, inflammation, and cardiac remodelling [96,101].

Echocardiography

Although echocardiographic findings are not specific to myocarditis, it remains a first-line diagnostic tool and is essential for disease monitoring [4,96]. However, its sensitivity is insufficient to definitively rule out myocarditis, necessitating further imaging modalities [102].

The main echocardiographic findings that can be observed in myocarditis include [3,103]:

- **Systolic and diastolic dysfunction** of the left ventricle or biventricular dysfunction, sometimes with regional wall motion abnormalities. Left ventricular ejection fraction (LVEF) at diagnosis serves as a strong prognostic marker, with lower values indicating a higher risk of adverse outcomes [13,25].
- **Left ventricular dilation**, which may not be present in early stages, as myocardial remodelling and dilation often develop later in the disease course [9,85,104].

- **Myocardial wall thickening and increased echogenicity**, indicative of myocardial oedema. In fulminant myocarditis, this often presents with a reduced ventricular cavity size and mild wall thickening due to extensive myocardial oedema [104].
- **Pericardial effusion**.
- Functional **atrioventricular valve regurgitation**, secondary to ventricular dilation and disfunction.

Speckle-tracking echocardiography provides additional diagnostic value beyond conventional echocardiography, particularly in patients with preserved LVEF. Global longitudinal strain reduction has been associated with the extent of myocardial oedema, making it a useful tool for early myocardial involvement assessment. However, its findings are not specific to myocarditis, as similar abnormalities are commonly observed in other forms of heart failure [104,105].

Microbiological testing

Despite the low diagnostic yield and lack of specificity, **serological testing** is still routinely performed in clinical practice, even though current guidelines do not recommend them due to their limited utility. Serologic tests for cardiotropic viruses in peripheral blood are rarely informative, except in cases where a specific infectious agent is strongly suspected; this is particularly important to consider since IgG antibodies against cardiotropic viruses are frequently detected in the general population, even in the absence of viral myocarditis. While serology may not confirm direct myocardial infection, it can indicate a previous or ongoing immune response to an infectious agent [4,96,106].

- **Polymerase chain reaction (PCR)** testing is available for the most common viral agents associated with myocarditis, including PVB19, adenovirus, enterovirus, Epstein-Barr virus, cytomegalovirus, and human herpesvirus-6. PCR can detect viral genomes in peripheral blood, respiratory secretions, or stool samples, identifying viral presence in approximately one-third of patients with suspected myocarditis. It is often used as a substitute for myocardial tissue PCR, despite its generally poor correlation with actual myocardial infection. Because of this, peripheral viral PCR should not replace myocardial PCR unless EMB is impossible to perform [4,9,107]. Only in infants with

PVB19 myocarditis, the blood PCR has shown a good correlation with myocardial PCR [24,108].

PCR testing in cardiac tissue remains the gold standard due to its high sensitivity and specificity, with the viral genome identified in approximately 45–50% of all suspected myocarditis cases. However, to exclude systemic infection, peripheral blood testing of peripheral blood is also recommended when EMB is performed [4,107].

- **Viral culture** from peripheral samples such as stool or urine is sometimes conducted in clinical practice. However, this method is unreliable in determining the aetiological cause, as the detection of a virus in non-cardiac tissues does not confirm it cause of myocarditis [9].

Positron emission tomography (PET)

With the widespread availability of CMR, the use of nuclear imaging techniques is not routinely recommended for the initial diagnostic evaluation of myocarditis. Several nuclear imaging techniques have been explored for detecting myocardial inflammation, however, their diagnostic accuracy is variable and generally lower compared to CMR. Positron emission tomography may be considered as an alternative in clinically stable patients who have contraindications for CMR, or in cases where there is suspicion of systemic autoimmune diseases affecting beyond the heart, particularly sarcoidosis, where it can help assess multiorgan involvement [1,4].

IX. ENDOMYOCARDIAL BIOPSY (EMB)

EMB remains the gold standard for diagnosing myocarditis, as it is the only method that allows both confirmation of myocardial inflammation and identification of the underlying aetiology [4,9,109]. However, despite its diagnostic value, EMB is significantly underutilized in clinical practice [1,77]. In paediatric patients, the diagnosis of myocarditis is often based solely on clinical findings without confirmatory examinations, as EMB is perceived as a high-risk procedure, and CMR often requires deep sedation in younger children [16,19,110]. Currently, the role of EMB and CMR in determining the aetiology of heart failure in neonates and infants remains poorly defined [9].

Regarding its indications, the European Society of Cardiology recommended in 2013 a routine use of EMB in the diagnostic process of myocarditis [4]. However, subsequent expert consensus statements suggest that EMB should be reserved for specific clinical scenarios where the findings could impact management or prognosis [83,111,112], or at least in situations where the prognostic and diagnostic value of the information outweighs the procedural risks [78]. A summary of the indications according to various clinical guidelines is provided in **table 3**.

ESC Position Statement (2013) [4]	**AHA Scientific Statement (2020)** [83]	**Expert Consensus Document (2020)** [1]	**ESC/HFSA/JHFA Trilateral Cooperation Project (2021)**[112]	**ACC Expert Consensus Decision Pathway (2024)** [78]
1. All clinically suspected myocarditis, regardless of clinical presentation. 2. Consider a repeat to monitor the response to targeted etiological treatment or in progressive HF if sampling error is suspected.	1.Acute unexplained cardiomyopathy with • Inotropes or MCS needed. • Sustained or symptomatic VT. • High grade AVB. • Lack of response to treatment within 1–2 weeks . 2. Suspected special conditions: sarcoidosis, eosinophilic, hydroxychloroquine-induced myocarditis.	1. Complicated myocarditis, including: • Fulminant myocarditis. • Acute HF. • VT. • High-grade AVB. 2. Special condition (ICI-associated and eosinophilic myocarditis). 3. Persistent or recurrent forms.	1. Complicated myocarditis, including: • Fulminant myocarditis. • Acute HF. • VT. • High-grade AVB. 2. To consider in clinically stable patients when myocarditis is suspected.	1. Stage B (asymptomatic) under ICI therapy. 2 Stage C (symptomatic) with • LV dysfunction • Symptomatic HF • Arrhythmia(high-degree AVB, VT) • Peripheral eosinophilia • Uncertain diagnosis and unable CMR 3. Stage D (advanced)

Table 3. Indications for endomyocardial biopsy. *Abbreviations:* MCS (mechanical circulatory support), ESC (European Society of Cardiology), AHA (American Heart Association), HFSA (Heart Failure Society of America), JHFS (Japanese Heart Failure Society), HF (heart failure), VT (ventricular tachycardia) AVB (atrioventricular block).

Given the variability in practice, it is crucial that each medical centre establishes its own protocol for EMB indications based on its institutional expertise and patient population [87], for example **Figure 5** provides a schematic overview of the diagnostic pathway in our centre.

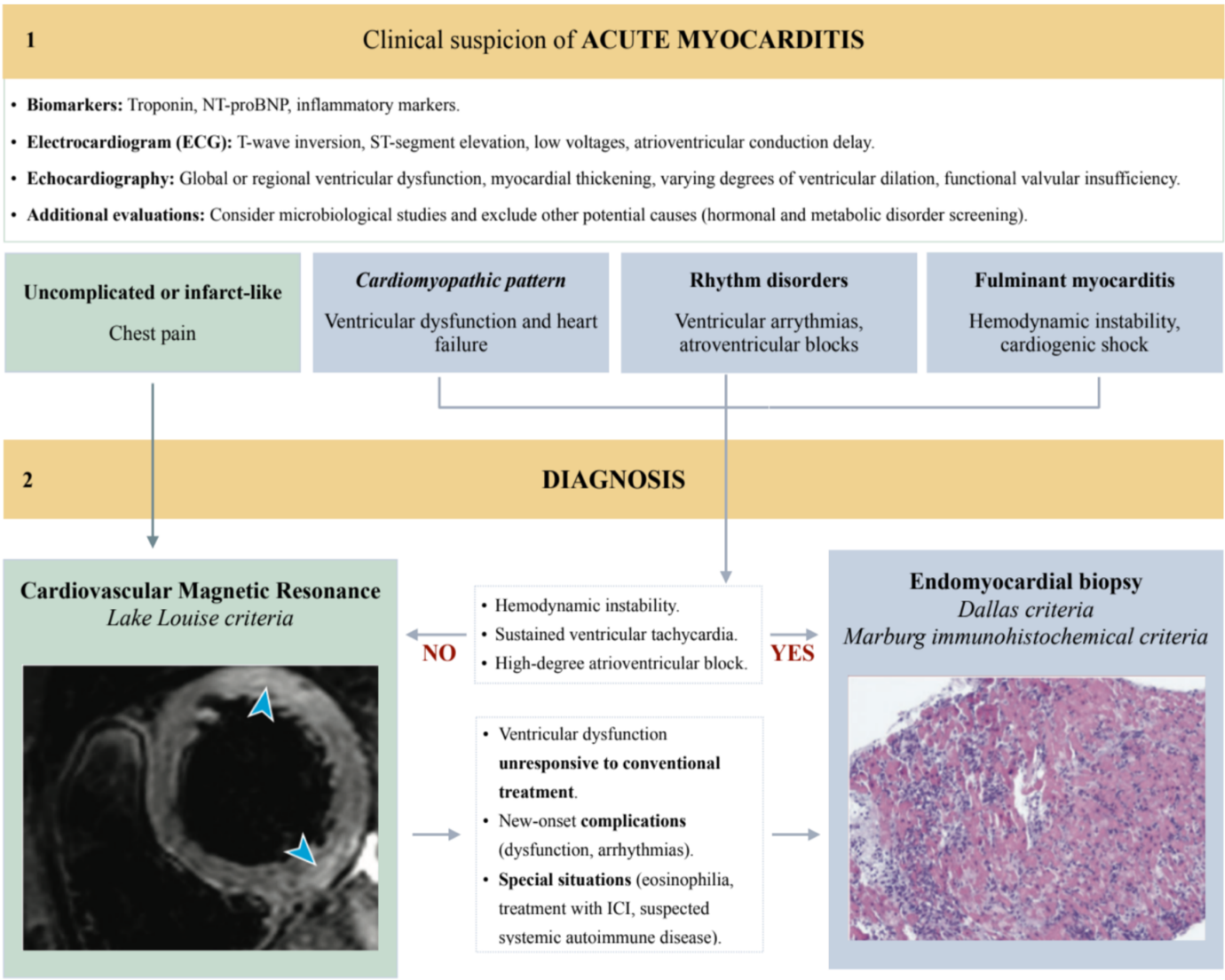


Figure 5. Overview of the diagnostic pathway for myocarditis.

Regarding prognosis, performing an EMB within two days of admission to the intensive care unit (ICU) was associated with improved survival free of heart transplantation or ventricular assist device implantation at one year, according to a propensity-matched, multicenter, retrospective cohort study [113].

The risk of complications in experienced centres is estimated at 1–2% in adults, with the most common adverse events including supraventricular arrhythmias, pericardial effusion, cardiac

tamponade due to myocardial perforation, valvular trauma, and transient atrioventricular block, in addition to local complications at the puncture site [109-112]. These risks are higher in patients with severe haemodynamic instability [11] and when EMB is performed in centres that infrequently perform EMB [78].

Given the focal and patchy nature of myocardial inflammation in myocarditis, a negative EMB result does not necessarily rule out the diagnosis [78]. To minimize sampling error and improve diagnostic sensitivity, it is recommended to obtain at least five biopsy samples [4,111,112]. Furthermore, inflammatory involvement in myocarditis predominantly affects the free wall of the left ventricle, an area that is inaccessible with standard EMB techniques, which typically obtain tissue from the right ventricle [109,112,114]. The diagnostic yield of EMB is highest when performed within the first two weeks of symptom onset [1,107].

When interpreting EMB results, it is important to recognize that myocardial inflammation is not specific to myocarditis and can also result from heart failure itself. Therefore, a global approach should be adopted in these situations, integrating CMR and EMB findings together with viral detection, laboratory biomarkers and genetic testing [110].

Different criteria have been proposed for the diagnosis of myocarditis,

1. **Dallas criteria**: Established in 1986, the Dallas criteria define myocarditis based on the histological triad of inflammatory infiltrates, adjacent myocyte degeneration, and non-ischemic necrosis. EMB findings can be classified as follows: [115]

    - Active myocarditis: both inflammatory infiltrates and myocyte necrosis and/or degeneration of adjacent myocytes.
    - Borderline myocarditis: inflammatory infiltrates without necrosis or degeneration of adjacent myocytes.
    - Absent myocarditis: no evidence of inflammation or necrosis.

    When comparing serial EMB samples, myocarditis can be further classified into persistent (ongoing), healing (resolving) and healed (resolved) myocarditis.

    Despite being widely used, its limitations include interobserver variability, lack of prognostic value, and low sensitivity, primarily due to sampling error in EMB [74,116].

2. **Marburg immunohistochemical criteria**: This approach enhances EMB sensitivity by using specific immunohistochemical markers for leukocytes (CD45), macrophages (CD68), T cells (CD3), T-helper cells (CD4), cytotoxic T cells (CD8), and B cells (CD19/CD20). Myocarditis is diagnosed when there is an infiltrate of ≥14 leukocytes/ mm², including up to 4 monocytes/mm², with a minimum of 7 CD3+ T cells/mm² [107,117]. These criteria provide greater sensitivity and superior diagnostic accuracy compared to the Dallas Criteria [117].

Currently, the Dallas criteria remain the most widely used histopathological standard for diagnosing myocarditis, with 80% of pathologists relying on them in routine practice. However, immunohistochemical techniques are an increasing adoption, with 40% of pathologists incorporating them to enhance diagnostic sensitivity and accuracy. A small proportion (8%) of pathologists do not utilize either of these methodologies, making it necessary to standardize protocols to improve consistency and reliability in myocarditis diagnosis. Among clinicians utilizing the Dallas criteria, achieving consensus on borderline myocarditis remains challenging, leading to increased variability in diagnostic criteria and limiting consistency in clinical interpretation [118].

Histopathological findings from EMB allow for the classification of myocarditis into distinct subtypes, as shown in **figure 6**, each associated with different underlying causes, prognostic significance and therapeutic implications [3,14,109].

1. **Lymphocytic myocarditis**: This is the most common histological type of myocarditis [3,13]. It can be associated with multiple aetiologies, including viral infections, drug reactions, and autoimmune diseases such as systemic lupus erythematosus, polymyositis/dermatomyositis, and rheumatoid arthritis. Histologically, EMB reveals irregular or diffuse inflammatory infiltrates primarily composed of lymphocytes and macrophages [1,76,118].

2. **Giant cell myocarditis**: Characterized by myocyte necrosis and multifocal or diffuse inflammatory infiltrates, consisting of T lymphocytes, multinucleated giant cells (derived from macrophages), and few eosinophils. While often idiopathic, it may also be associated with autoimmune disorders (14-20%). Giant cell myocarditis carries a poor prognosis, and the key objective of EMB is to rule out this histological subtype due to its aggressive course and need for early immunosuppressive treatment [13,119].

3. **Granulomatous myocarditis (sarcoidosis-associated myocarditis)**: Defined by the presence of noncaseating granulomas with macrophages and multinucleated giant cells, surrounded by fibrosis and lymphocytic infiltrates. This subtype of myocarditis in is highly suggestive of cardiac sarcoidosis [2,117].

4. **Eosinophilic myocarditis**: Characterized by a predominantly eosinophilic interstitial inflammatory infiltrate, often without significant myocyte damage. It is frequently associated with peripheral eosinophilia due to drug hypersensitivity, parasitic infections, Churg-Strauss syndrome (eosinophilic granulomatosis with polyangiitis), or endomyocardial fibrosis [72].

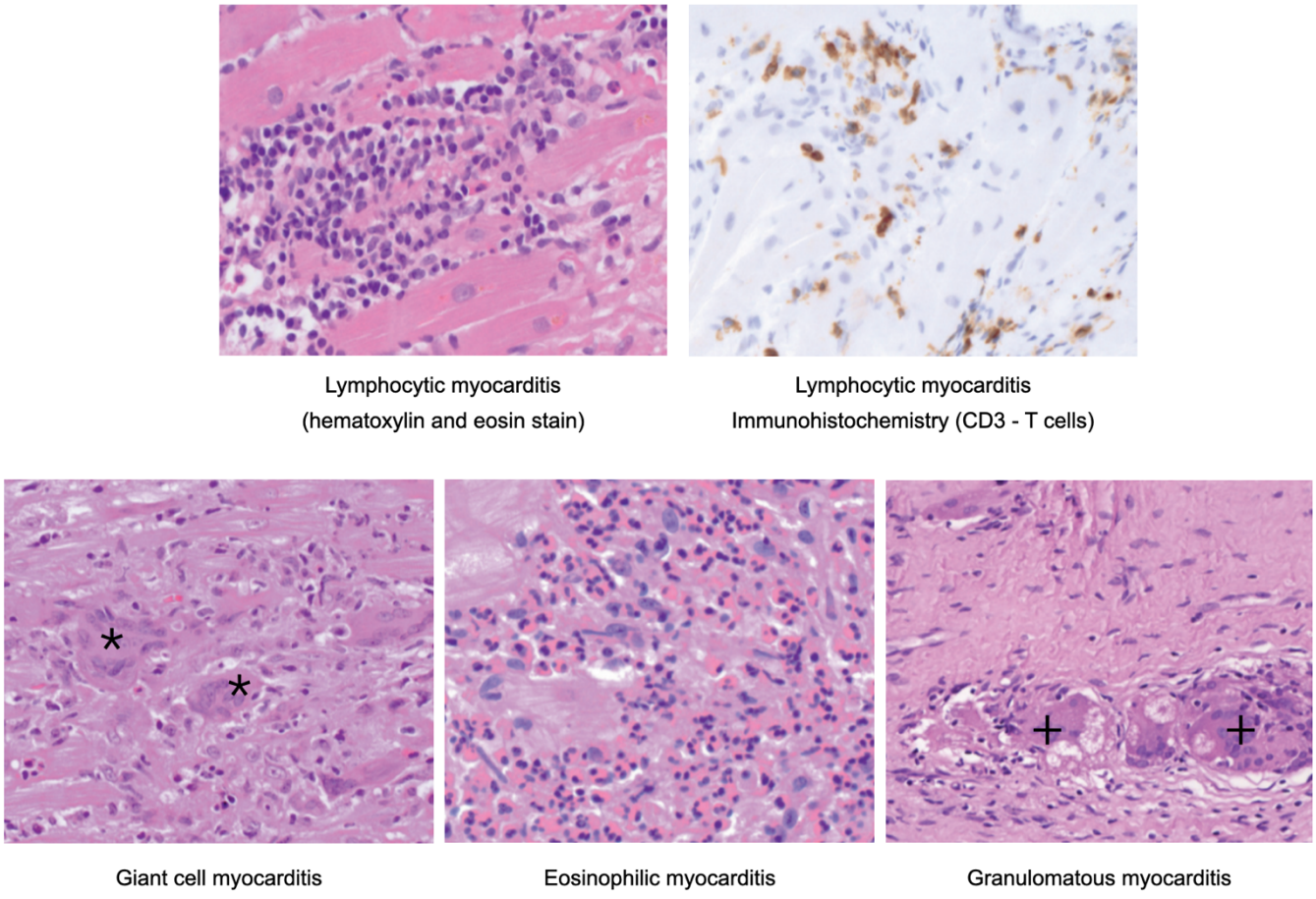


Figure 6. Histopathological subtypes of myocarditis
(*Adapted from: J Am Coll Cardiol. 2025;85(4):391-431*) [78].

A. Lymphocytic myocarditis is characterized by diffuse mononuclear infiltrates.
B. Eosinophilic myocarditis is defined by eosinophilic granulocytes and macrophages.
C. Giant cell myocarditis with mononuclear infiltrates, multinucleated giant cells (*) and eosinophils.

D. Sarcoidosis-associated (granulomatous) myocarditis with granulomas (+) and the absence of necrotic myocytes.

The detection of viral genome in cardiac tissue using quantitative PCR is a highly sensitive and a quite specific method in the paediatric population, available for major viral pathogens associated with myocarditis, and is a complement to immunohistological analysis [9,114]. On the other hand, its diagnostic value in adults seems to be more limited for certain virus, such as PVB19, as viral genome detection in myocardial tissue may represent residual presence from a past infection rather than active myocarditis [35,120].

Current evidence does not support it as a routine test, as most available data come from small studies with inconclusive results, primarily performed in patients with chronic myocarditis [2,6,121]. Moreover, scientific societies differ in their recommendations regarding its application [112]. While the European Society of Cardiology advocates for the routine implementation of viral genome detection in myocarditis evaluation [4], the *American Heart Association* considers it a potentially useful option, but only in cases where diagnostic uncertainty remains after standard evaluation [83]. Some centres do not have familiarity or access to it and they are less commonly performed in the United States than in Europe [78].

X. CARDIOVASCULAR MAGNETIC RESONANCE IMAGING (CMR)

CMR is a non-invasive diagnostic tool that enables detailed tissue characterization, including the quantification of myocardial inflammation and fibrosis [3,122]. While it does not replace EMB, it is considered a valuable alternative in clinically stable patients [4] and has been designated as a Class 1 indication in recent recommendations[78]. However, in complicated forms of myocarditis, CMR should not substitute EMB, as histological analysis remains essential for definitive diagnosis and aetiology-specific therapeutic decision-making [4,111,121]. Additionally, CMR is often challenging to perform in haemodynamically unstable patients due to high heart rates, potential arrhythmias, and mechanical ventilation dependency, all of which can affect image quality and acquisition feasibility [2,122].

CMR is considered the *gold standard* for the quantification of ventricular volumes, LVEF, and myocardial mass [9,122]. It also allows for the detection of key markers of inflammation and necrosis. In patients with myocarditis, CMR typically reveals the presence of irregular

subepicardial oedema with variable intramyocardial extension [85,87,123]. Myocardial oedema tends to decrease approximately four weeks after disease onset, therefore, for optimal diagnosis accuracy, CMR should be performed within the first 2–3 weeks [1,123].

Myocardial oedema is primarily assessed using T2-based techniques (T2-weighted imaging and T2 mapping), though T1 mapping has also proven effective in detecting oedema and myocardial inflammation. Hyperaemia and increased capillary permeability contribute to extracellular space expansion, which can be quantified through early gadolinium enhancement (EGE) imaging. Additionally, it can be assessed with extracellular volume (ECV) mappings, calculated from pre- and post-contrast T1 values. Myocyte necrosis and fibrosis are detected using late gadolinium enhancement (LGE) imaging, which in myocarditis typically presents as midwall or subepicardial involvement, predominantly affecting the lateral and inferior walls, though subendocardial involvement may also be observed [124,125]. While LGE patterns are not pathognomonic for a specific aetiology of myocardial inflammation, recognizing certain distribution patterns can guide further diagnostic evaluation. For instance, ring-like midwall or subepicardial LGE patterns have been associated with desmoplakin-related and other genetic cardiomyopathies, highlighting the role of genetic testing in these cases [60].

The Lake Louise Criteria, first published in 2009 as part of an international consensus, consisted of three main criteria: regional high T2 signal intensities on T2-weighted images or increased global T2 signal intensity ratio, increased early gadolinium enhancement ratio on T1-weighted images, and areas with high signal intensities in nonischemic distribution pattern on LGE images. These criteria allowed for a probable diagnosis of myocarditis when at least two out of three criteria were met, with a sensitivity of 74% and specificity of 86% [102,126,127].

In 2018, the Lake Louise Criteria were updated to incorporate tissue mapping techniques, significantly enhancing diagnostic accuracy. These consist of two main criteria (T1-based criterion and T2-based criterion). T1-based criterion is considered to be positive if increase of native T1 relaxation times, increase of ECV, or positive LGE exist. T2-based criterion is positive in cases of increased T2 relaxation times or in cases with regional or global high T2 signal intensities on T2-weighted images [122]. These updates have increased diagnostic sensitivity to 87.5% and specificity to 96.2% in adult populations [117,128], primarily due to the improved performance of native T1 mapping [126,129]. Under the revised criteria, the presence of a single positive criterion is now sufficient to confirm myocarditis in patients with clinical

suspicion, but with less specificity, suggesting that added caution is needed in such scenarios. Signs of pericarditis and ventricular systolic dysfunction act as supportive criteria. [122]. **Figure 7** is a representation of the original and the 2018 Lake Louise Criteria.

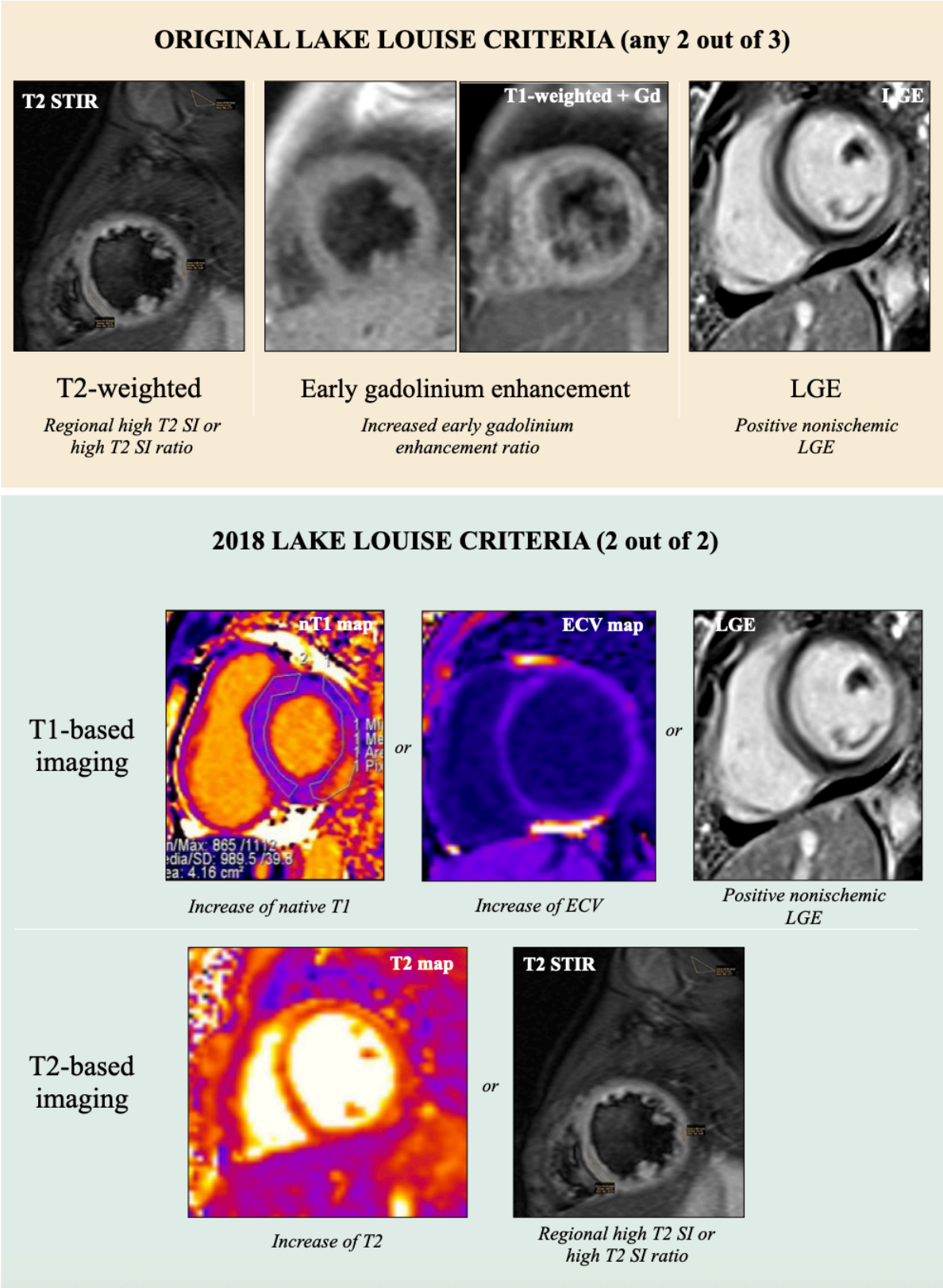


Figure 7. Illustration of the original and 2018 Lake Louise criteria for CMR in myocarditis [128], using representative examples from patients included in our cohort. *Abbreviations:* Gd (gadolinium), SI (signal intensity), STIR (short tau inversion recovery).

A comparison of the original and the 2018 Lake Louise Criteria is presented in **table 4**.

Lake Louise Criteria (2009)	Updated Lake Louise Criteria (2018)
1. Hyperintensity on T2-weighted imaging (oedema). 2. Early gadolinium enhancement (hyperaemia). 3. Late gadolinium enhancement (necrosis or fibrosis).	1. T1-weighted imaging (non-ischemic necrosis or fibrosis). One of the following: • Increased native T1 mapping. • Increased extracellular volume. • Late gadolinium enhancement. 2. T2-weighted imaging (myocardial oedema). One of the following: • Increased T2 mapping. • Hyperintensity on T2-weighted imaging.

Table 4. CMR diagnostic criteria for myocarditis [122].

The diagnostic sensitivity of CMR varies depending on clinical presentation. Its accuracy is highest in infarct-like myocarditis, while it is relatively lower in patients with cardiomyopathic or arrhythmic presentations [130]. The high diagnostic yield of CMR has also been demonstrated in paediatric populations, although data remain limited due to smaller studies and variability in imaging acquisition protocols across different centres [131,132].

Beyond its diagnostic utility, CMR also plays a key role in patient follow-up, typically performed 6 to 12 months after diagnosis to assess for persistent fibrosis and residual inflammation [1]. Persistent myocardial changes are relatively common in paediatric patients [133]. Additionally, the persistence of fibrosis without associated oedema is recognized as a poor prognostic marker, particularly when fibrosis is localized in the mid-wall of the

interventricular septum, which has been linked to adverse long-term outcomes [134,135] (example in **figure 8**).

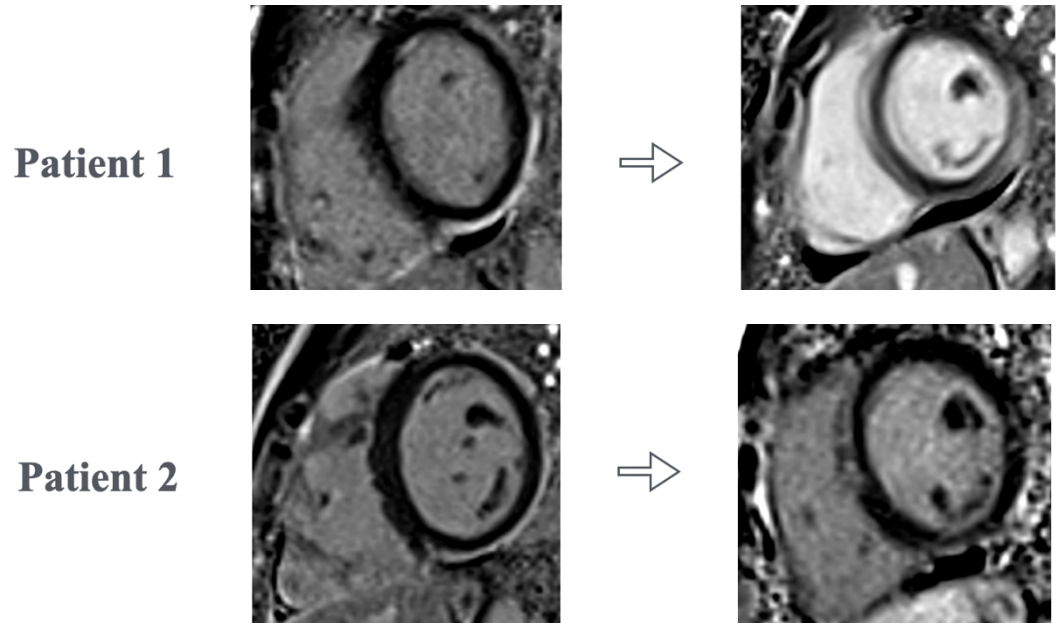


Figure 8. CMR images from two paediatric patients initially diagnosed with uncomplicated myocarditis, both showing LGE predominantly affecting the lateral wall of the left ventricle. At 12-month follow-up, patient 1 demonstrated progression of LGE into a ring-like pattern, suggestive of evolving myocardial involvement, while patient 2 showed a marked reduction in LGE, indicating resolution of myocardial injury.

XI. GENETIC TESTING

The relationship between myocarditis and genetic cardiomyopathies has been well-documented, as discussed earlier in this text. However, routine genetic testing for patients with myocarditis is not yet universally recommended in clinical practice [56]. Despite this, there is growing evidence to support targeted genetic testing in specific cases, particularly in familial forms of myocarditis, recurrent myocarditis, or when imaging findings suggest an underlying cardiomyopathy, typically DCM or NDLVC [55,57,63]. Genetic evaluation may also be warranted in patients with a history of ventricular arrhythmias or a family history of sudden cardiac death [71,136].

Recently, a consensus statement from the *Spanish Society of Cardiology (Sociedad Española de Cardiología, SEC)* proposed an algorithm for identifying adult patients who should undergo

genetic testing [137]. Notably, our research has confirmed the relevance of these risk factors in paediatric population (**figure 9**). Genetic testing is primarily recommended for individuals with a family history of myocarditis, cardiomyopathy, or sudden cardiac death before the age of 50, as well as those with recurrent myocarditis or sustained ventricular tachycardia. Additionally, genetic evaluation may be considered in cases where, at six-month follow-up CMR, there is persistent extensive LGE affecting more than three myocardial segments, LGE patterns associated with poor prognosis (septal or ring-like), LVEF < 40%, or persistent troponin elevation [137]. These risk factors have been observed significantly more frequently in genetically positive myocarditis patients compared to gene-negative cases. Consequently, these clinical and imaging features could serve as red flags, prompting genetic testing in patients at higher risk for myocarditis recurrence and ventricular tachyarrhythmias [49,59].

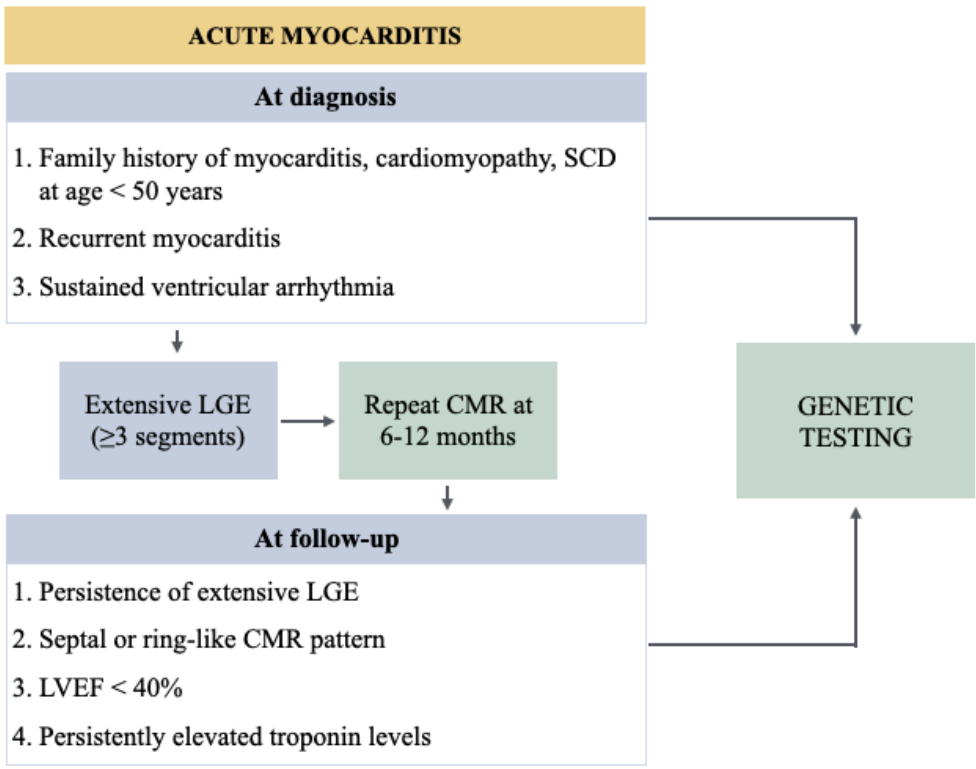


Figure 9. Recommendations for genetic testing in myocarditis according to the *Spanish Society of Cardiology* (*Adapted from: Rev Esp Cardiol, 2024;77(8):667-79)* [137].
Abbreviations: SCD (sudden cardiac death).

Given the complexity and heterogeneity of myocarditis, an individualized diagnostic approach is essential, selecting the most appropriate genetic testing modality based on clinical

presentation, associated conditions, and family history. **Table 5** outlines the various genetic testing methods available, along with their respective strengths and limitations [138,139]. In most centres, next-generation sequencing (NGS) panels are the preferred method, as they allow for the targeted identification of disease-associated variants within a well-established set of genes linked to cardiomyopathy phenotypes [139]. Some of the key genes prioritized in genetic studies include: *ACTC1, BAG3, DES, DSC2, DSG2, DSP, EMD, FLNC, JUP, MYBPC3, MYH7, PKP2, PLN, TMEM43, LMNA, RBM20, SCN5A, TNNC1, TNNI3, TNNT2, TPM1*, and *TTN* [137]. However, to improve diagnostic accuracy and research comparability, a standardized and validated myocarditis-specific gene panel should be established, likely incorporating genes linked to dilated and arrhythmogenic cardiomyopathies [48].

Test Type	Indications	Advantages	Limitations
Sanger Sequencing	SNVs in specific single-gene disorders, known familial variants. Confirming results from other tests	High accuracy and specificity for single genes. Low cost per gene	Low throughput, does not detect large deletions or duplications. Limited to known variants
Next-generation sequencing	Higher diagnostic yield, simultaneous and high-throughput sequencing of a lot of DNA and RNA molecules. Reduces cost and time for sequencing.		
Panel Sequencing	When there is a well-established group of genes associated with a phenotype	Simultaneous analysis of tens to hundreds of genes. Detection of both SNVs and small insertions/deletions.	Limited to known genes, may miss novel or non-coding variants
WES	Complex or atypical presentations, syndromic features, undiagnosed cases	Broad scope, captures novel and unexpected coding (exonic) variants	Misses non-coding and structural variants, high cost, potential for incidental findings

WGS	Non-diagnostic cases after WES, complex phenotypes	Comprehensive analysis, detects non-coding (intronic) and coding variants	High cost, complex interpretation, potential for incidental findings
Non-sequencing approaches			
aCGH	Unexplained developmental delays, suspected large CNVs	Detects large chromosomal imbalances and CNVs	Does not detect SNVs or small insertions/deletions
MLPA	Suspected gene deletions or duplications	Targeted detection of specific deletions/duplications	Limited to known deletions/ duplications, does not detect point variants
FISH	Suspected known chromosomal abnormalities	Detects specific chromosomal abnormalities such as translocations	Limited resolution, targeted to specific regions, cannot detect small variants
Karyotyping	Suspected large chromosomal changes	Detects large-scale chromosomal abnormalities such as trisomies or translocations	Low resolution, does not detect smaller genetic changes like SNVs

Table 5. Comparative overview of genetic tests. *Abbreviations:* SNVs (single nucleotide variants), NGS, (Next-generation sequencing), WES (Whole Exome Sequencing), WGS (Whole Genome Sequencing), aCGH (Array Comparative Genomic Hybridization), MLPA (Multiplex Ligation-dependent Probe Amplification), FISH, (Fluorescence in Situ Hybridization), CNVs (copy number variations).

Finally, genetic counseling for cardiomyopathies is already guideline-recommended in other myocardial diseases. Given the increasing recognition of genetic predisposition in acute myocarditis, similar recommendations should be considered for selected patients with high-risk clinical features [48].

XII. GENERAL MANAGEMENT

Hospital admission is always recommended for patients with suspected myocarditis due to the unpredictable nature and the potential for rapid progression to a complicated form of the disease [4,9]. Some authors even advocate for immediate ICU admission in all cases, needing continuous haemodynamic monitoring, given the risk of sudden clinical deterioration [140]. The decision for ICU admission should be individualized based on clinical presentation, the presence of ventricular dysfunction, arrhythmias, or biomarker elevation, and the need for advanced circulatory support.

Currently, there are no specific evidence-based management considerations for paediatric patients affected by acute myocarditis, and existing evidence is primarily extrapolated from studies on the treatment of heart failure and arrhythmias [6,19,140]. The therapeutic approach is dictated by disease severity, clinical presentation, and underlying aetiology, beginning with the classification of patients into complicated and non-complicated forms [3]. **Figure 10** provides a schematic overview of the recommended management strategies.

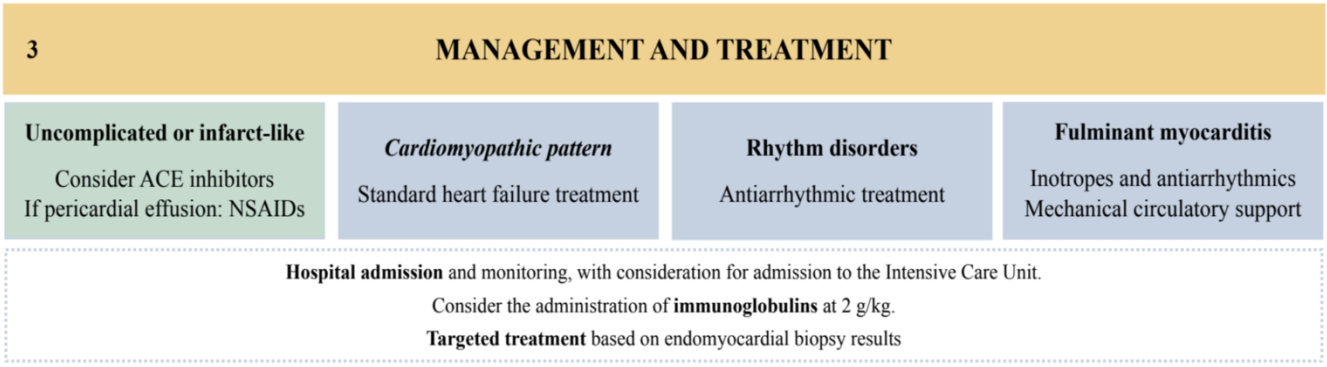


Figure 10: Overview of management strategies. This figure is a continuation of **Figure 5**.

Non-complicated myocarditis (infarct-like presentation)

Patients with mild, infarct-like myocarditis may often be managed conservatively, with nonsteroidal anti-inflammatory drugs (NSAIDs) considered in cases with pericardial effusion [3,141]. In these patients, CMR can detect subclinical myocardial injury and fibrosis, which may not be apparent on standard cardiac assessments such as echocardiogram. Therefore, it is crucial in identifying patients with subclinical myocardial injury and fibrosis, who might benefit from therapies aimed at preventing adverse ventricular remodelling, such as angiotensin-converting enzyme inhibitors (ACEI). However, the optimal duration of

treatment remains uncertain, as myocardial damage, even without evident dysfunction, may predispose patients to progressive remodelling and further cardiomyopathy [109].

Cardiomyopathic pattern (heart failure presentation)

For patients presenting with ventricular dysfunction and heart failure, standard heart failure therapy is recommended, incorporating anti-congestive treatment with diuretics alongside neurohormonal inhibition strategies aimed at preventing ventricular remodelling. The heart failure therapy includes ACEI, beta-blockers, mineralocorticoid receptor antagonists (MRA) and angiotensin receptor-neprilysin inhibitors (ARNI). These therapies have been demonstrated to improve prognosis, reduce mortality, and lower the risk of heart transplantation in patients with haemodynamic stability [9,88,116].

Oral heart failure therapy should commence once the patient has stabilized beyond the acute phase and continues to exhibit systolic dysfunction or heart failure. When inotropic support is necessary, oral therapy should be introduced concurrently with the weaning process to ensure a smooth transition and optimize cardiac function [109].

Although clinical data are limited, sodium-glucose cotransporter 2 inhibitors (SGLT2I) have shown potential cardioprotective effects in myocarditis [142]. Experimental studies have demonstrated that Dapagliflozin reduces the severity of Coxsackievirus B3 myocarditis, improves cardiac function, and enhances survival by modulating inflammatory responses [143]. Similarly, Canagliflozin and Empagliflozin have shown anti-inflammatory benefits in experimental autoimmune myocarditis models [142]. However, as these findings are derived from animal studies, their clinical relevance in human myocarditis remains to be established.

Arrhythmic presentation

Currently, there are no specific antiarrhythmic therapies for myocarditis-related arrhythmias. Management is based on general antiarrhythmic principles, with close monitoring for potential ventricular arrhythmias, high-degree atrioventricular block, or malignant tachyarrhythmias [82].

Fulminant Myocarditis (cardiogenic shock)

Evidence-based treatment strategies for fulminant myocarditis remain limited, and

management primarily follows general protocols for cardiogenic shock [121].

First-line therapy involves inotropic support to maintain cardiac output and organ perfusion. However, excessive doses may increase myocardial oxygen demand, potentially worsening myocardial recovery [87]. Milrinone is typically the first-line inotrope, due to its vasodilatory and inotropic effects, while vasopressor-inotropes like adrenaline and dopamine are generally reserved for refractory hypotension, given their pro-arrhythmic potential and excessive chronotropic effects [9,16,87].

Despite aggressive medical management, 14-23% of paediatric patients require mechanical circulatory support, most commonly ECMO [19,144]. This complex situation is often observed in younger children (0-2 years), with ECMO primarily used as a bridge to recovery [1,83,87]. The goals of mechanical circulatory support in fulminant myocarditis include:

- Optimization of systemic and coronary perfusion.
- Biventricular unloading to reduce myocardial workload.
- Venous decongestion to prevent or reverse multi-organ dysfunction associated with low cardiac output states [145].

However, ECMO is not without risks. It can increase left ventricular afterload and can also potentially worsen myocardial inflammation and adverse remodelling [2]. Multicentre studies report short-term transplant-free survival rates of 66-76% in paediatric patients requiring ECMO [3,86].

If ECMO cannot be successfully weaned after 2-3 weeks, transition to long-term mechanical circulatory support should be considered, and the need for heart transplantation should be evaluated [1,9].

XIII. AETIOLOGY-SPECIFIC TREATMENTS

Immunosuppressive therapy

At present, there is insufficient scientific evidence to universally recommend immunosuppression in the management of myocarditis [1,9]. Most studies have not demonstrated a significant difference in terms of mortality or need for heart transplantation between patients who receive immunosuppressive therapy and those who do not [16,146,147].

However, some reports suggest that immunosuppressive treatment may be associated with improved cardiac function, particularly in cases refractory to standard haemodynamic support or chronic heart failure therapy [148–150].

Given the controversial results from the limited studies available, and the possibility of spontaneous recovery without intervention, the role of immunosuppression remains a topic of debate. Additionally, many of the studies supporting its use include both acute and chronic inflammatory cardiomyopathies, often with longer disease durations and rarely involving fulminant presentations [148-153].

Some researchers believe that fulminant myocarditis may have similar mechanisms to acute cellular rejection after heart transplantation, as they share key pathophysiological mechanisms. Based on this concept, immunosuppressive regimens similar to those used for transplant rejection have been explored, particularly in the most severe cases [13,121].

Current recommendations suggest that immunosuppression should be considered when viral infection is ruled out via EMB [1,4,6] and in the context of autoimmune disorders or isolated cases with specific histological findings [121,154] (**Figure 11**). In situations other than these, each centre can decide based on its experience [9]. However, in complicated myocarditis, urgent immunosuppression may be considered even before EMB results are available, particularly in fulminant cases [1,83,155].

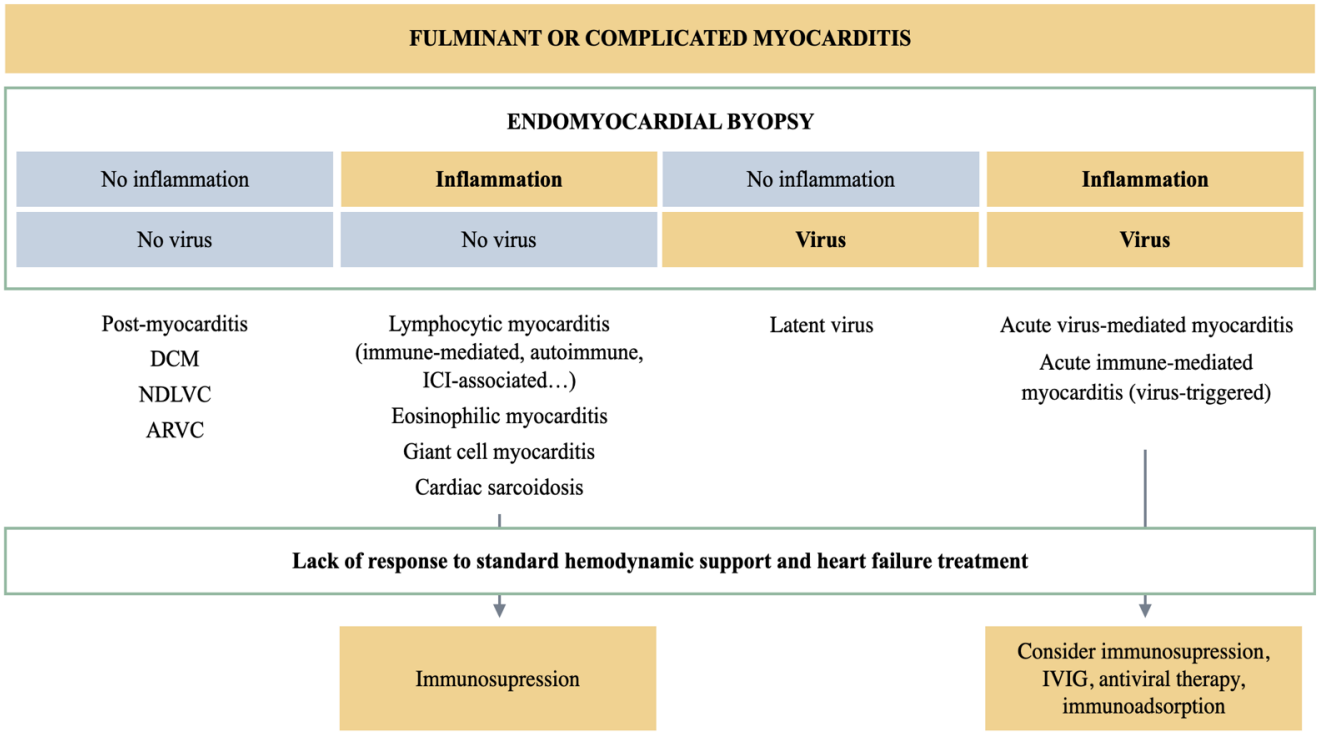


Figure 11. Therapeutic options for complicated myocarditis based on EMB findings. *Abbreviations*: DCM (dilated cardiomyopathy), NDLVC (non-dilated left ventricular cardiomyopathy), ARVC (arrhythmogenic right ventricular cardiomyopathy), IVIG (intravenous immunoglobulins).

Although some clinical guidelines contraindicate the use of immunosuppressive drugs in the presence of viral infection in myocardial tissue [4], it is well established that immune-mediated mechanisms are the primary drivers of myocardial injury in viral myocarditis [2]. Thus, modulating the inflammatory cascade could be beneficial in select cases [154,156]. This is particularly relevant for PVB19-associated myocarditis, where both direct viral damage and immune-mediated injury are implicated [28,31]. Importantly, immunosuppressive therapy does not appear to enhance viral replication in these cases [156]. Additionally, the detection of PVB19 in EMB may represent latent infection rather than active myocarditis, as the virus is frequently found incidentally in asymptomatic individuals [31]. Consequently, the mere presence of a viral genome in myocardial tissue should not serve as an absolute contraindication to immunosuppressive therapy, and some patients may still benefit from its use. However, this rationale does not apply to coxsackievirus-induced myocarditis, where direct viral cytotoxicity is the dominant mechanism and persistent viral replication exacerbates myocardial damage [11,27,31]. Immunosuppression should also be avoided in immunocompromised patients [1].

- **Corticosteroids**: The first-line immunosuppressive agents and are used empirically in approximately 25% of paediatric myocarditis cases [16,19]. However, their role remains controversial [3], and their clinical impact is still uncertain [16,121,157].

  A 2015 Cochrane systematic review evaluated the efficacy of corticosteroids in randomized controlled trials involving both paediatric and adult patients, including a total of 719 participants (200 of whom were paediatric). The review concluded that while corticosteroids do not improve survival or reduce the need for transplantation, they are associated with a significant improvement in myocardial function [158]. In complicated myocarditis, empirical corticosteroid administration is often recommended [56,121,155].

- **Other immunosuppressive agents (mycophenolate, cyclosporine, azathioprine, methotrexate)**: Although not standardized, the administration of low-dose corticosteroids in combination with a steroid-sparing immunosuppressant may be

beneficial in patients with persistent myocardial inflammation or ongoing injury (persistence of troponin release) [2,148].

Intravenous immunoglobulin (IVIG)

Although there is insufficient evidence to support IVIG administration in myocarditis [1,6], its immune-modulating and anti-inflammatory properties make it a commonly used therapy in paediatric patients with complicated myocarditis, either with myocardial dysfunction or with arrhythmias [16,19,87]. While multiple studies have failed to demonstrate a clear survival benefit [159-161], some favourable reports are based on small sample sizes or historical controls [146,162].

Although the experience in adults is limited, IVIG is frequently used in pediatric lymphocytic myocarditis [87] as it has no major side-effects [4]. It may be used in lymphocytic myocarditis refractory to conventional heart failure therapy, both viral and autoimmune forms, particularly if autoantibody-mediated [4], administered at a dose ranging from 0.5 g to 1 g/kg, with evidence of some benefits in terms of functional recovery and survival [83,159].

IVIG is not thought to be immunosuppressive but it has anti-inflammatory, antiviral, and immunomodulatory effects and is considered safe [9]. It has been shown to increase levels of anti-inflammatory mediators, which may contribute to ventricular function improvement and enhanced survival in chronic heart failure, independent of aetiology [163].

Immunoadsorption

Various autoantibodies have been identified in myocarditis, particularly in cases with an autoimmune aetiology, suggesting a potential pathogenic role in disease progression. Consequently, therapeutic approaches commonly employed in other autoimmune disorders, such as immunoadsorption, have been explored as potential treatment strategies. Immunoadsorption aims to eliminate circulating cardiotoxic autoantibodies and pro-inflammatory cytokines, thereby mitigating immune-mediated myocardial injury [4]. Emerging evidence suggests that this therapy may contribute to improved myocardial function in patients with heart failure by reducing inflammation and modulating the immune response [164,165].

Antiviral therapy

Although the efficacy of antiviral therapy in myocarditis has not been rigorously established,

its known benefits in non-cardiac viral infections suggest that it should be considered in cases of active viral infection, with minimal side effects [2,9].

- **Interferon-β (IFN-β):** is a cytokine primarily secreted by immune system cells, particularly fibroblasts, participating in antiviral defense and immune regulation. In patients with myocarditis, IFN-β levels are often diminished, suggesting a potential deficiency in the host antiviral response [166].

  A six-month IFN-β therapy has demonstrated efficacy in chronic cardiomyopathies associated with enterovirus or adenovirus infections, promoting viral clearance from the myocardium, reducing disease progression, and improving ventricular function [166]. Early initiation of IFN-β therapy has been correlated with better long-term survival rates, particularly when administered before irreversible myocardial damage [39]. Additionally, beneficial effects have been reported in PVB19-associated cardiomyopathy [167] and in case series of patients with PVB19-related myocarditis [24,149].

  However, the presence of PVB19 genomes in myocardial tissue does not always imply an active pathogenic role in adults. PVB19 can persist in solid tissues for years following the initial infection, even in healthy, immunocompetent individuals. In such cases, the detection of PVB19 DNA may be an incidental finding rather than the primary driver of cardiomyopathy, suggesting that additional contributing factors may underlie myocardial dysfunction in these patients [35,120].

- **Other antiviral agents**: While these agents are effective in their respective viral infections, their efficacy in myocarditis remains unproven [4].

  - Acyclovir (herpes simplex virus)
  - Ganciclovir/valganciclovir (cytomegalovirus, human herpesvirus 6)
  - Oseltamivir (influenza virus)
  - Cidofovir (adenovirus)
  - Remdesivir (SARS-CoV-2)
  - Antiretroviral therapy (human immunodeficiency virus)

To sum up, in complicated myocarditis, initial management should prioritize immunosuppressive therapy. Subsequently, based on histopathological findings from EMB, the treatment regimen should be adjusted accordingly, as shown in **figure 12**. A personalized, pathology-driven approach may optimize outcomes and minimize disease progression [3,11,121].

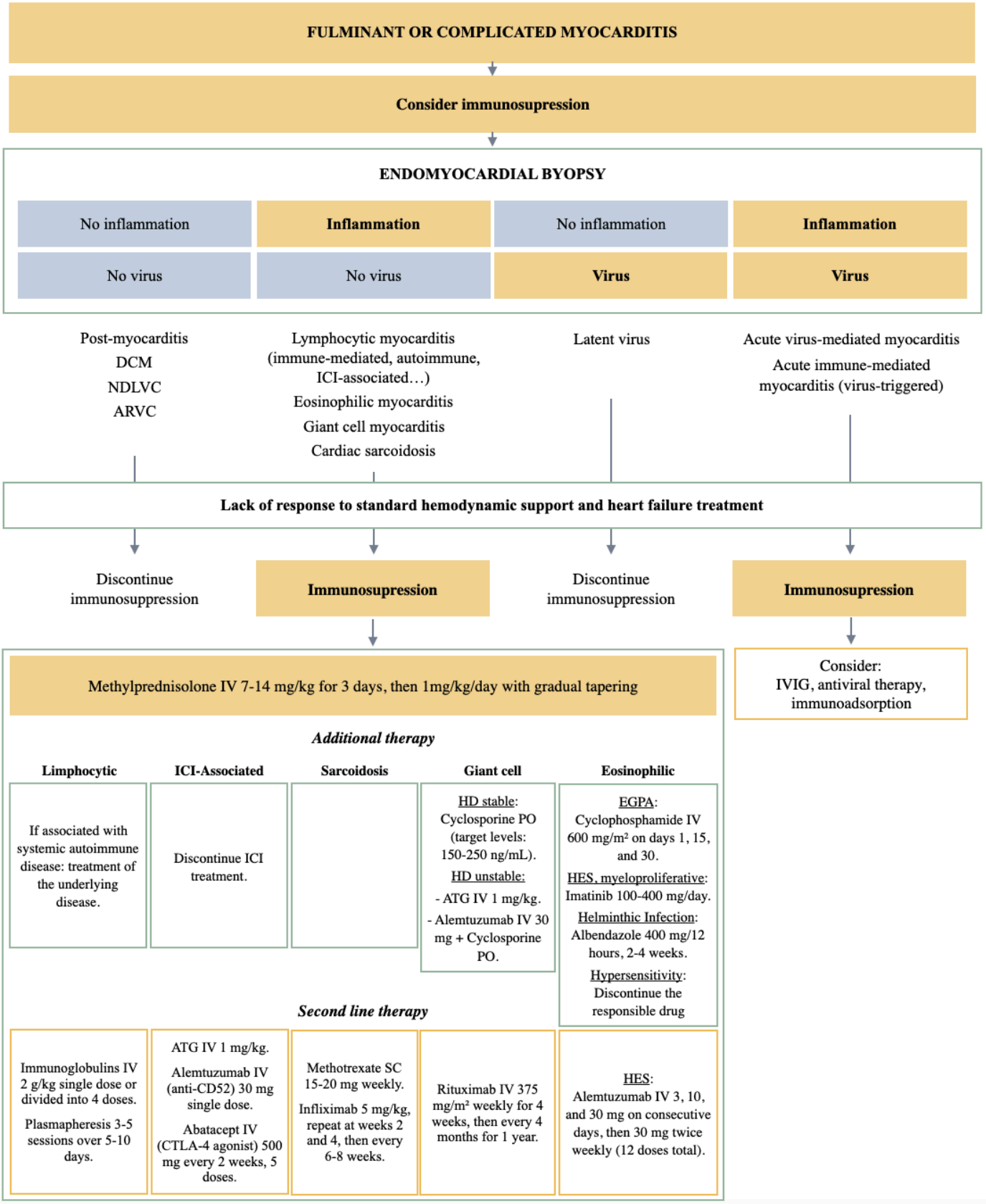


Figure 12. Therapeutic options for complicated myocarditis based on EMB findings [1,3,5].

Abbreviations: EV (intravenous), PO (oral administration), SC (subcutaneous), ICI (immune checkpoint inhibitors), HD (haemodynamically), ATG (anti-thymocyte globulin), EGPA (eosinophilic granulomatosis with polyangiitis), HES (hypereosinophilic syndrome), DRESS (Drug Rash with Eosinophilia and Systemic Symptoms).

XIV. FOLLOW-UP STRATEGIES

Long-term follow-up is strongly recommended for patients who have experienced an episode of acute myocarditis [4]. The initial follow-up examinations should include ECG, transthoracic echocardiography and laboratory testing with cardiac biomarkers (particularly if values remain elevated beyond the acute phase), at a frequency similar to that required for recently diagnosed acute heart failure in the context of any cardiomyopathy [9].

Additionally, an exercise stress test is often advised before resuming sportive activity, typically around six months after the acute episode. This test helps assess exercise tolerance, detects any residual myocardial dysfunction that may not be evident at rest, and evaluates the presence of exertion-induced arrhythmias [1,9]. Given the potential for subclinical myocardial fibrosis and electrical instability post-myocarditis, a stress test provides additional safety when determining an individual's readiness to return to sports, particularly in competitive [168].

The Spanish Society of Cardiology (Sociedad Española de Cardiología, SEC) recommends that patients with myocarditis may be stratified into three distinct risk categories based on their clinical evolution and diagnostic findings [137]. According to their recommendations, follow-up strategies should be individualized to each patient's risk profile to optimize long-term outcomes.

Table 6 shows a proposed follow-up schedule according to the patient's risk category. Although these follow-up recommendations are primarily based on adult studies and guidelines, there is no clear consensus regarding the optimal duration of follow-up in the paediatric population. Despite this uncertainty, our centre currently adopts a strategy of maintaining regular cardiology follow-up until patients reach adulthood, with the aim of early detection of potential long-term sequelae and ensuring a safe transition to adult cardiology care.

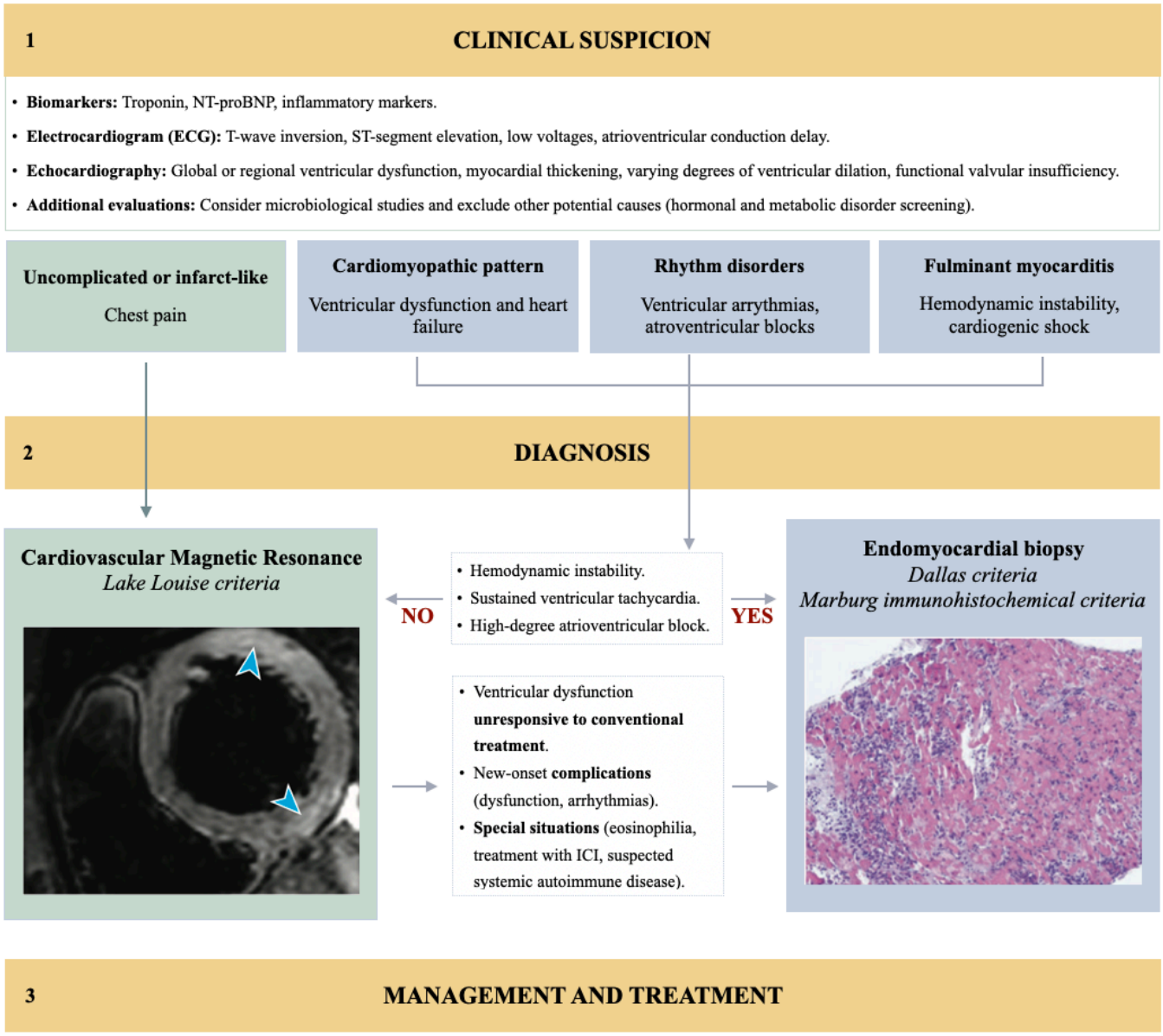
	Low risk	Intermediate risk	High risk
HD	Stable	Stable	Unstable or heart failure symptoms.
ECG	Nonspecific changes (ST or T wave abnormalities)	Nonspecific changes (ST or T wave abnormalities)	
Arrhythmia	No complex arrhythmias	No complex arrhythmias	Complex arrhythmias (high-grade AV block or SVT)
LVEF	Preserved. No regional wall motion abnormalities	35-50%, with or without regional wall motion abnormalities	Severely depressed (<35%)
CMR	Oedema present Minimal or absent LGE	LGE, especially involving the interventricular septum	Extensive LGE (≥3 segments) or high-risk pattern (septal or ring-like)
Recommended follow-up	Clinical assessment at 3-6 months ECG and blood tests If normal, discharge to primary care If persistent abnormalities, escalate to intermediate-risk protocol	Clinical assessment at 3, 6 and 12 months ECG and blood tests Echocardiogram at 6 months CMR at 12 months Discharge or annual follow-up according to follow-up CMR results Exercise stress test before returning to sport	Clinical assessment at 3, 6 and 12 months ECG and blood tests Echocardiogram and holter every 3-6 months or annually based on results CMR at 6 months Exercise stress test before returning to sport EMB at 3 months if aetiology remains unclear and no improvement

Table 6. Risk stratification and recommended follow-up protocol for patients with acute myocarditis (adapted from the Spanish Society of Cardiology protocol (*Adapted from: Rev Esp Cardiol, 2024;77(8):667-79)* [137].
Abbreviations: SVT (sustained ventricular tachycardia).

There is ongoing debate regarding the necessity of follow-up EMB or CMR. From a practical standpoint, if ventricular function remains impaired or biomarkers continue to be abnormal,

repeating an EMB or CMR is a reasonable approach. In cases where the episode appears resolved, a follow-up CMR at 6 to 12 months can help assess the persistence of residual oedema or fibrosis [1,9].

Figure 13 provides a comprehensive overview of the diagnostic approach, therapeutic strategies, and follow-up considerations in myocarditis, as implemented in our centre and as outlined in the previous sections. This algorithm integrates current clinical guidelines and evidence-based recommendations for a structured evaluation and management of affected patients.



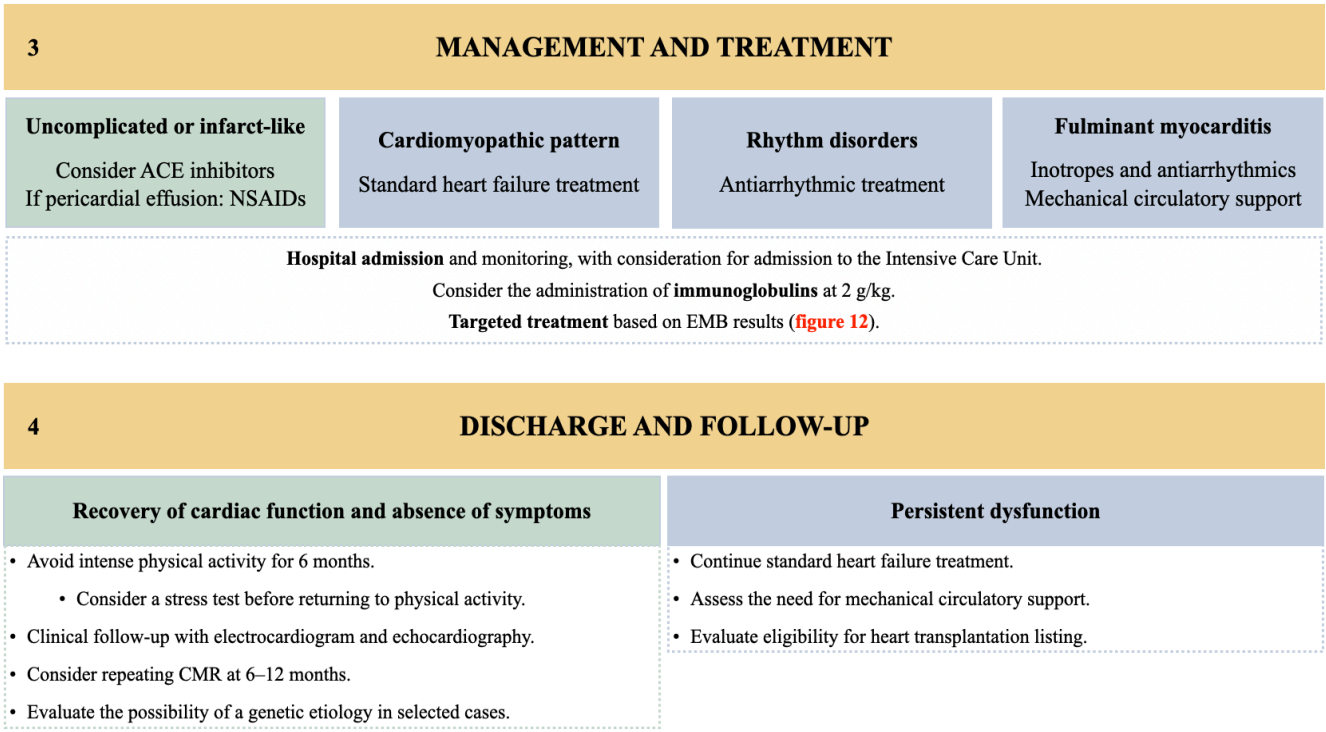


Figure 13: Representation of the diagnosis, management and follow-up [3,4,9,78].

Sports recommendations

Regarding physical activity recommendations, exercise is strictly contraindicated during the acute phase of myocarditis, as it can precipitate arrhythmias and exacerbate myocardial injury. It should be restricted for at least six months, regardless of the initial severity of the episode [6,168,169]. Moreover, episodes of myocarditis have been associated with sudden cardiac death during physical activity, even in patients with preserved systolic function and irrespective of the degree of myocardial inflammation [75]. The decision to resume sports participation, after at least six months of restriction, should be based on the normalization of cardiac function and biomarker levels [169,170]. Available data suggest that the risk of cardiac events during exercise is considered low one year after the diagnosis of myocarditis [171].

XV. PROGNOSIS

Paediatric patients with myocarditis can experience either partial or complete spontaneous clinical recovery. However, in some cases, the disease may persist subclinically and progress to DCM in up to 30% of adult patients [4]. The overall risk of mortality or heart transplantation

in paediatric patients ranges from 2% to 13%, with the highest risk observed in younger children [16,19,144].

The prognosis of myocarditis largely depends on the underlying aetiology and the initial clinical presentation pattern [25,115]. Adult patients with an uncomplicated presentation typically have a favourable prognosis, with mortality rates close to 0% [77]. In contrast, those presenting with arrhythmias, ventricular dysfunction or heart failure symptoms face a significantly worse prognosis, both in the short and long term, with an overall mortality or transplantation risk reaching up to 48% [13,25,85]. Initial systolic dysfunction has been identified as the strongest predictor of adverse outcomes, as well as the need for ECMO [25]. In paediatric patients, ECMO is associated with a mortality rate of 66% in paediatric patients [15].

In patients with complicated myocarditis presenting with arrhythmias, adult cohorts have reported a recurrence rate of arrhythmic events ranging from 30-37% [80-81]. Furthermore, studies have demonstrated an increased risk of sudden cardiac death and ventricular arrhythmias in patients who initially presented with sustained ventricular tachycardia, fibrosis affecting two or more myocardial segments and the absence of oedema on the initial CMR [80].

Additional factors associated with poor prognosis include the presence of giant cell histology, a prolonged QRS duration greater than 120 ms on the initial electrocardiogram [13], and myocardial fibrosis, particularly in the anteroseptal region of the left ventricle [2,172,173] as fibrosis in this location may involve the conduction system, creating a substrate for malignant arrhythmias [172].

Although myocarditis is generally considered an acute and self-limiting condition, some cases may relapse years after the initial episode. While recurrence remains rare, most documented cases have been reported in adults [24,174,175]. However, as previously discussed, recent evidence suggests that recurrent myocarditis may serve as an early clinical indicator of an underlying genetic predisposition [65,66]. In particular, patients with P/LP variants in cardiomyopathy-associated genes appear to be at increased risk of repeated inflammatory episodes [54,55]. These recurrent events may reflect the so-called “hot phases” of, in which myocardial inflammation precedes overt structural abnormalities [62-64]. In fact, some patients with recurrent myocarditis are later diagnosed with inherited cardiomyopathies, reinforcing the need for continuous follow-up, early identification of at-risk individuals and the implementation of personalized long-term management strategies.

RATIONALE FOR THE STUDY

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Paediatric myocarditis represents a clinically heterogeneous condition, encompassing a wide range of manifestations that span from mild, infarct-like episodes to fulminant presentations with cardiogenic shock and multi-organ failure. Despite major advances in diagnostic techniques, including CMR, EMB, and molecular virology testing, the early identification of high-risk patients and personalized treatment strategies remain a significant clinical challenge. Outcomes in paediatric myocarditis are highly variable, and clinicians currently lack reliable tools to predict the evolution of the disease at the time of diagnosis.

One of the key unmet needs in this field is the ability to identify, from the initial stages, clinical, imaging, or molecular markers that can inform prognostic stratification and guide management decisions. From a clinical standpoint, distinguishing between patients who will recover spontaneously and those who may develop complications such as recurrent myocarditis, chronic left ventricular dysfunction, or progression to dilated cardiomyopathy is essential. This is particularly relevant in children and adolescents, where early myocardial injury may have lifelong consequences. In the acute setting, it is crucial to determine whether a patient presenting with new-onset heart failure is experiencing a transient, reversible episode (often mediated by infection) or whether the underlying process is a chronic, genetically mediated cardiomyopathy that may ultimately require heart transplantation.

In recent years, growing evidence has focused on to two principal factors contributing to the pathogenesis and progression of myocarditis: the role of viral and immune-mediated mechanisms, and the contribution of genetic susceptibility. Viral pathogens can act either as primary drivers of myocardial inflammation or as secondary triggers in genetically predisposed individuals, initiating a "second-hit" phenomenon. Simultaneously, P/LP variants in genes associated with cardiomyopathies, such as desmosomal or sarcomeric proteins, have been increasingly recognized in patients initially diagnosed with myocarditis. In these cases, the identification of myocardial inflammation may reflect an early “hot phase” of an inherited cardiomyopathy, with inflammation acting as the first clinical manifestation of a progressive genetic disease. These findings challenge the traditional paradigm of myocarditis as an exclusively acquired, self-limited disease and introduce the concept of an overlapping spectrum between inflammatory and genetic myocardial disorders.

Nevertheless, despite these advances, current clinical and imaging criteria often fail to clearly distinguish virus-mediated myocarditis from early-stage genetic cardiomyopathies. This diagnostic challenge is particularly pronounced in the paediatric population, where data are limited and study cohorts tend to be small and heterogeneous. Consequently, there is an increasing need to improve our diagnostic approach and algorithms, integrating clinical presentation, advanced imaging techniques, molecular testing, and genetic profiling to better define the underlying pathophysiology in each patient.

The clinical implications of early identification of the underlying mechanism of myocarditis are substantial. In cases of virus-mediated myocarditis, prompt recognition of the etiological agent could enable targeted antiviral or immunomodulatory therapy, potentially limiting myocardial damage and prompting faster and more complete recovery of cardiac function.

In contrast, early identification of a genetic basis for the disease would lead to a fundamentally different management approach, shifting the focus toward long-term disease management. This includes the need for cascade family screening to identify at-risk relatives, the adoption of individualized recommendations regarding sportive activities, and consideration of arrhythmic risk stratification, including the possible need for implantable cardioverter-defibrillators (ICDs). Moreover, establishing a genetic diagnosis early on time, may inform long-term surveillance strategies, reproductive counselling, and lifestyle modifications not only for the affected child but also for family members who may carry the same P/LP variants.

Ultimately, improving our understanding of the diverse aetiologies and risk factors associated with paediatric myocarditis will help transition from a one-size-fits-all model to a precision medicine approach. This would not only optimize the acute management and long-term outcomes of affected children but also reduce the burden of late-stage complications through earlier and more accurate intervention.

HYPOTHESIS

HYPOTHESIS

1. **A substantial proportion of paediatric patients diagnosed with uncomplicated myocarditis carry P/LP variants in cardiomyopathy-associated genes**, even in the absence of family history or prior cardiac disease. Suggesting the presence of an underlying cardiomyopathic substrate initially masked by inflammatory features, with important implications for long-term management and prognosis.

2. **Genetic testing plays a pivotal role in the diagnostic evaluation of paediatric patients with new-onset heart failure**. When systematically integrated into clinical practice, alongside clinical assessment, imaging findings and histopathological analysis, it supports a more accurate aetiological diagnosis.

3. **Standard diagnostic tools, such as the 12-lead electrocardiogram, may offer early indicators of specific viral aetiologies,** particularly PVB19. It could facilitate faster diagnostic orientation and support the use of targeted treatments in selected cases.

4. **Cardiac magnetic resonance imaging performed at the time of presentation may not reliably differentiate between virus-mediated myocarditis and genetically mediated cardiomyopathies in infants presenting with acute heart failure. Similarly, the presence of myocardial inflammation detected by endomyocardial biopsy and defined by the Marburg immunohistochemical criteria may not be specific for myocarditis.** The overlap in inflammatory findings reinforces the need for adjunctive diagnostic modalities, such as quantitative histological and genetic analysis, to clarify disease aetiology.

5. **Ongoing clinical and imaging follow-up is essential for all paediatric patients diagnosed with myocarditis, including those with uncomplicated forms.** Although CMR performed at diagnosis may not identify the underlying aetiology, longitudinal follow-up may unmask evolving myocardial dysfunction, recurrence, or genetic markers of disease progression, thereby informing prognosis and guiding long-term surveillance and therapeutic decisions.

OBJECTIVES

OBJECTIVES

1. To characterise the clinical presentation, diagnostic work-up, and outcomes of paediatric patients diagnosed with acute myocarditis, with a focus on identifying early prognostic indicators.

2. To evaluate the role of viral infections, particularly PVB19, in paediatric myocarditis and investigate whether specific electrocardiographic patterns can support early aetiological orientation.

3. To determine the prevalence and relevance of P/LP variants in cardiomyopathy-associated genes among paediatric patients with uncomplicated myocarditis.

4. To assess the diagnostic utility and limitations of cardiac magnetic resonance imaging and endomyocardial biopsy in guiding diagnosis and differentiating between virus-mediated myocarditis and early-stage genetic cardiomyopathies. In this context, to investigate whether specific radiological patterns identified on cardiac magnetic resonance imaging may serve as early indicators of an underlying genetic background in paediatric patients affected with myocarditis.

5. To explore the value of systematic genetic testing, in conjunction with imaging and histology, for improving the aetiological diagnosis of inflammatory cardiomyopathies in paediatric patients; thereby supporting the integration of clinical, imaging, histological, and genetic data into more precise diagnostic and management pathways for paediatric myocarditis.

6. To highlight the importance of structured follow-up in patients with myocarditis, including those with initially uncomplicated disease, for the detection of late-onset abnormalities or progression to cardiomyopathy.

METHODOLOGY

METODOLOGY

I. STUDY DESIGN

This was a prospective, descriptive, multicentre study conducted over a 16-year period (2008–2024), including consecutive paediatric patients (≤18 years of age) admitted with a diagnosis of myocarditis.

The study was centralized at *Vall d'Hebron Hospital Campus*, with the collaboration of paediatric cardiology units from eight hospitals in Catalonia, including:

- *Hospital de la Santa Creu i Sant Pau* (Barcelona)
- *Hospital Universitari Doctor Trueta* (Girona)
- *Hospital Universitari Sant Joan de Reus* (Reus)
- *Hospital Universitari Joan XXIII* (Tarragona)
- *Hospital General de Granollers* (Granollers)
- *Consorci Sanitari de Terrassa* (Terrassa)
- *Consorci Corporació Sanitària Parc Taulí* (Sabadell)
- *Hospital Germans Trias i Pujol* (Badalona)

Additionally, data were collected from three hospitals in other regions of Spain:

- *Hospital Virgen de la Arrixaca* (Murcia)
- *Hospital Universitario Araba* (Basque Country)
- *Hospital Universitari Son Espases* (Balearic Islands)

II. CLINICAL DIAGNOSIS AND INCLUSION CRITERIA

Myocarditis was diagnosed according to the *European Society of Cardiology (ESC)* criteria [4], and the 2021 *American Heart Association (AHA)* Scientific Statement on myocarditis in children [9] based on the presence of a compatible clinical presentation, including new-onset heart failure, cardiogenic shock, arrhythmias, or severe precordial pain, with confirmation either by EMB or by CMR, meeting the 2018 Lake Louise criteria [122].

Patients with congenital structural heart defects, syndromic disorders, or metabolic, mitochondrial, or neuromuscular diseases were excluded from the study.

The study flowchart, detailing follow-up procedures is presented in **figure 14.**

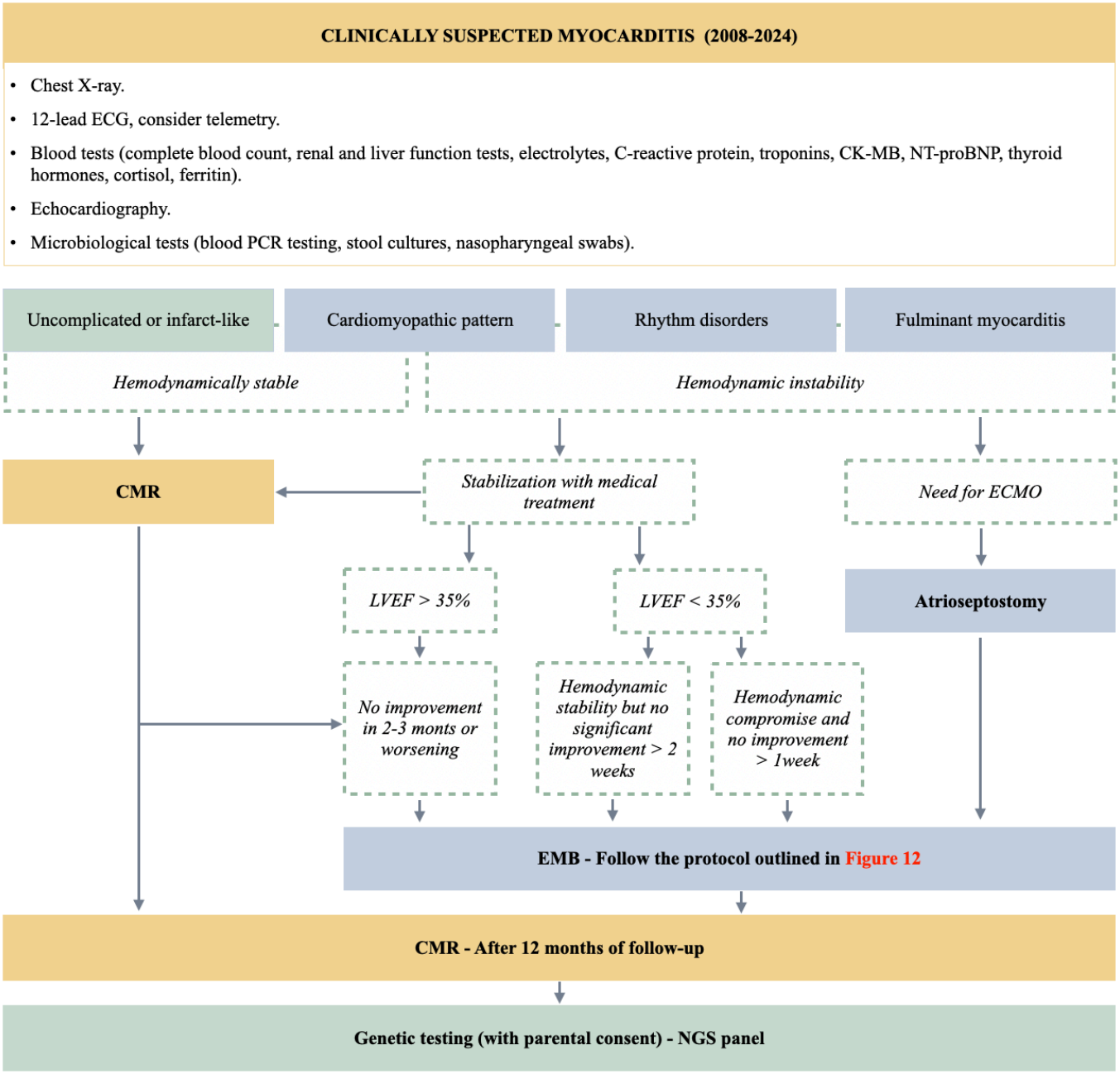

Figure 14. Study flowchart illustrating the clinical pathway for patients diagnosed with acute myocarditis, including decision points for performing EMB and CMR.

III. INITIAL INVESTIGATIONS

Clinical and epidemiological data were collected, with a particular focus on family history of heart transplantation, sudden cardiac death, or unexplained cardiomyopathy, given their potential role in identifying inherited or genetic predispositions.

All patients admitted with suspected myocarditis underwent a standardized set of initial investigations. Further details on the expected findings for each investigation are discussed in the Introduction section.

Blood tests

All patients diagnosed with myocarditis underwent comprehensive blood work, including complete blood count, renal and liver function tests, electrolytes, C-reactive protein, and cardiac injury biomarkers (troponins, creatine kinase-MB, and NT-proBNP). Additionally, at admission, thyroid hormones, cortisol, ferritin, and copper levels were assessed to rule out other systemic conditions.

Chest X-ray

A chest X-ray was performed at the time of diagnosis to assess cardiomegaly and pulmonary congestion. Follow-up imaging was conducted to evaluate disease progression and potential complications, including pulmonary oedema.

Electrocardiogram and telemetry

A 12-lead ECG was obtained at admission and repeated during hospitalization as needed. Continuous telemetry monitoring was performed in all patients admitted to the ICU and in those requiring close rhythm surveillance during hospitalization.

Echocardiography

All echocardiographic studies were performed by specialized paediatric cardiologists. The initial echocardiogram was conducted at the referring hospital, and if the patient was transferred to a tertiary care centre, follow-up studies were performed by paediatric specialists at the referral institution (Vall d'Hebron Hospital Campus). The echocardiographic parameters were assessed using equipment from General Electric (Vivid S70, Vivid-I, Vivid E95) and Philips (Epiq CVx, Affiniti 70). The following echocardiographic parameters were analysed:

- Chamber dimensions, measured in the parasternal long-axis view at end-diastole (left ventricle, left atrium, and right ventricle). Measurements exceeding two standard deviations above normal values for body surface area were considered pathological.

- Interventricular septal thickness and left ventricular posterior wall thickness, measured in the parasternal short-axis view at end-diastole. Values exceeding two standard deviations above normal were considered hypertrophy.
- Left ventricular function, assessed using the biplane Simpson method and Teichholz method. Normal values were defined as ≥50% for Simpson's method and ≥55% for Teichholz, with dysfunction classified as mild (45–55%), moderate (35–45%), or severe (<35%).
- Right ventricular function, assessed using tricuspid annular plane systolic excursion (TAPSE), tricuspid regurgitation severity, and fractional area change (FAC), with normal FAC defined as ≥35%.
- Tissue Doppler imaging (TDI), evaluating e', a', and S wave velocities at the mitral annulus, interventricular septum, and tricuspid annulus, as well as E/e' ratio to estimate left ventricular filling pressures.
- Atrioventricular valve regurgitation, assessed for severity.
- Pericardial effusion, evaluated for presence and extent.
- Speckle-tracking echocardiography, performed during follow-up to provide additional prognostic information, particularly in patients with preserved ejection fraction.

Microbiological tests

To determine the underlying aetiology, blood PCR testing was performed for enterovirus, adenovirus, parvovirus B19, cytomegalovirus, Epstein-Barr virus, herpes simplex virus 1 and 2, human herpesvirus 6, HIV, Mycoplasma pneumoniae, and Toxoplasma gondii. Additional PCR testing was conducted based on the clinical context, along with stool cultures for bacterial and viral pathogens and nasopharyngeal swabs for respiratory viruses.

IV. CARDIOVASCULAR MAGNETIC RESONANCE (CMR)

CMR was performed within the first two weeks after disease onset, once patients had achieved initial stabilization and were clinically stable enough to be transferred to the radiology department. **Figure 14** represents the flowchart outlining the selection criteria for CMR in this cohort.

All CMR studies were conducted using a 1.5-T Magnetom Avanto scanner (Siemens Medical System, Erlangen, Germany) with software releases VB17 and VE11B, employing cardiac synchronization for optimized imaging. Steady-state free precession (SSFP) cine sequences were acquired in a short-axis stack to evaluate biventricular function and volumes. T2-weighted short-tau inversion recovery (T2W-STIR) and T1-weighted turbo spin-echo (TSE) sequences were obtained both before and after intravenous administration of gadolinium-based contrast. LGE imaging was performed using phase-sensitive inversion recovery (PSIR) sequences, acquired 10 minutes after administration of 0.15 mmol/kg gadolinium, to assess myocardial fibrosis and necrosis.

Quantitative tissue characterization was performed using T1 and T2 mapping techniques, measuring native T1 (nT1), extracellular volume fraction (ECV), and T2 relaxation times. These measurements were taken at the left ventricular posterior wall and interventricular septum in end-diastole, using short-axis views. T1 mapping was performed with a modified Look-Locker inversion recovery (MOLLI) sequence, both pre-contrast and 15 minutes post-contrast, while T2 maps were acquired during an 11-second end-expiratory breath-hold to minimize respiratory motion artefacts.

To assess regional myocardial abnormalities, the 17-segment left ventricular model standardized by the *American Heart Association (AHA)* was utilized [176].

The 2018 revised Lake Louise criteria were applied for the diagnosis of myocarditis, as described in the introduction section [122]. Additionally, a follow-up CMR study was performed at 12 months to evaluate the persistence of myocardial involvement and assess long-term cardiac recovery in affected patients.

V. ENDOMYOCARDIAL BIOPSY (EMB)

EMB was performed in patients ≥6 months of age and weighing ≥8 kg, as it is considered a safe in this population when conducted by experienced operators [111, 177]. Candidates for biopsy presented with new-onset ventricular dysfunction of unknown aetiology and met at least one of the following criteria:

a. Fulminant myocarditis with hemodynamic shock requiring ECMO. In these cases, left ventricular decompression via percutaneous atrial septostomy was routinely performed in our centre for patients with severe ventricular dysfunction requiring ECMO.

b. Left ventricular ejection fraction <35% with hemodynamic compromise and no echocardiographic improvement after ≥1 week of medical treatment.

c. Left ventricular ejection fraction <35% with hemodynamic stability, but no significant improvement after ≥2 weeks of medical treatment.

For infants <6 months of age or weighing <8 kg, the decision to perform an EMB was made on an individualized basis, considering the clinical context and procedural risk. **Figure 14** illustrates the decision-making algorithm and procedural workflow for EMB.

EMB was performed via percutaneous jugular vein access, using a 6Fr flexible bioptome under fluoroscopic and echocardiographic guidance to ensure safe and accurate tissue sampling. Six biopsy specimens were obtained from the mid-to-basal portion of the right interventricular septum, a site chosen for its lower risk of perforation and higher diagnostic yield due to the typical distribution of myocardial inflammation [109,112]. All biopsies were performed in a cardiac catheterization laboratory with continuous hemodynamic and electrocardiographic monitoring to detect potential complications. After the procedure, patients remained under strict post-procedural observation.

Biopsy specimens underwent histopathological and molecular evaluation, including:

- Standard histology: Haematoxylin–Eosin and Masson's trichrome staining to assess inflammatory infiltrates, myocardial necrosis, and fibrosis.
- Immunohistochemistry: Staining for CD45 (leukocytes), CD20 (B cells), CD3 (T cells), and CD68 (macrophages) to characterize immune cell infiltration patterns.
- Molecular testing: PCR for viral genome detection.

Inflammatory cardiomyopathy was diagnosed according to the Marburg immunobiological criteria, defined as ≥14 mononuclear cells per mm², including ≥7 T-lymphocytes per mm² [107,117].

VI. GENETIC ANALYSIS

Although genetic testing was not traditionally performed in patients with myocarditis, routine genetic evaluation has been progressively implemented since 2021. Additionally, retrospective genetic analysis was carried out in previously collected prospective cases of infarct-like myocarditis.

Genetic testing was carried out across two specialized institutions: *Vall d'Hebron Hospital Campus* and *Hospital de la Santa Creu i Sant Pau*, each employing distinct sequencing strategies to investigate genetic contributors to myocarditis and inherited cardiovascular diseases.

Vall d'Hebron Hospital Campus

At Vall d'Hebron, genomic DNA was extracted from peripheral blood using standard procedures. It was analysed using the TruSight Cardio Panel (Illumina), which applies next-generation sequencing (NGS) to target a curated set of 174 genes implicated in hereditary cardiac conditions. Library preparation was conducted using TruSight reagents, ensuring comprehensive enrichment of coding regions and adjacent intronic boundaries, thereby increasing the likelihood of detecting clinically significant variants. Sequencing was performed on an Illumina MiSeq platform.

All variants identified through NGS underwent further validation via Sanger sequencing, a gold-standard technique that enhances accuracy by confirming the presence of detected variants and reducing the risk of false-positive results.

Hospital de la Santa Creu i Sant Pau

In this centre, DNA libraries were constructed using SeqCap or KAPA kits (Roche) and enriched via custom clinical exome probes (CES, Roche) that cover approximately 5,000 genes associated with monogenic diseases, as defined by OMIM and selected literature. The design also included capture of known pathogenic deep intronic variants listed in ClinVar [178] at the time of development.

Sequencing was performed on Illumina NextSeq platforms (NextSeq500 or NextSeq1000), generating paired-end reads of 2 × 150 bp or 2 × 100 bp, respectively. Quality control was assessed using Picard tools (http://broadinstitute.github.io/picard/), with the following metrics

obtained: mean on-target coverage of 111.3×, median coverage of 106.6×, and 98.6% of target bases covered at ≥20×.

Sample identity was verified through DNA fingerprinting, comparing 14 SNPs genotyped via custom MLPA (Multiplex Ligation-dependent Probe Amplification) assays.

Bioinformatics

Raw sequencing data (FASTQ files) were processed through a standardized bioinformatics pipeline, including alignment to the GRCh37 human reference genome using the BWA algorithm [179], followed by variant calling with the Genome Analysis Toolkit (GATK) [180]. Annotated variant calls were generated using a combination of custom scripts and public databases. Copy number variant (CNV) analysis was performed using the ExomeDepth package in R [181].

For the diagnostic evaluation, a virtual gene panel tailored to the suspected cardiomyopathy phenotype was applied to prioritize clinically relevant findings.

Variant classification

Variant classification incorporated a multi-step approach involving:

- Population allele frequency data from gnomAD [182], Kaviar [183] and a local in-house dataset.
- Variant consequence (e.g., missense, splicing, frameshift, nonsense, CNV).
- Functional prediction tools such as REVEL for pathogenicity scoring [184]
- Databases including ClinVar [178] and Human Gene Mutation Database (HGMD) [185].
- Review of current literature and integration with the patient's clinical data.

The classification of genetic variants was conducted following the *American College of Medical Genetics and Genomics (ACMG)* guidelines [186] and the recommendations of the Clinical Genome Resource (ClinGen) Sequence Variant Interpretation Working Group [187]. A multidisciplinary team composed of cardiologists, clinical and laboratory geneticists and genetic counsellors reviewed final candidate variants to assess pathogenicity in the context of the patient's phenotype.

Co-segregation

When feasible, segregation analysis in first-degree relatives was performed using Sanger sequencing to assess the inheritance pattern of candidate variants and strengthen the genotype-phenotype correlation.

VII. FOLLOW-UP

From the time of diagnosis, all patients were scheduled for regular follow-up visits, with the frequency of evaluations tailored to the severity of the initial presentation and the presence of ongoing symptoms. Clinical assessments included cardiac function monitoring, symptom progression evaluation, and treatment adjustments as necessary.

As part of the standardized protocol, a follow-up CMR was performed 12 months after the initial episode to assess for persistent myocardial involvement, fibrosis, or functional abnormalities. The findings helped guide long-term management and optimize risk stratification for potential late complications. Subsequent follow-up was further individualized based on CMR findings, clinical status, and long-term cardiac function recovery.

VIII. ETHICS

Ethical approval for this study was obtained from Vall d'Hebron ethics committee (PR(AMI)43/2019), ensuring strict adherence to international ethical guidelines and the protection of participants' rights, dignity, and safety. All research activities were conducted in compliance with the principles outlined in the 1975 Declaration of Helsinki and its subsequent revisions, which establish the ethical framework for medical research involving human subjects [188]. The study design, data collection procedures, and patient management protocols were reviewed to guarantee ethical integrity and minimize any potential risks associated with participation.

Confidentiality and data protection were upheld throughout the study, with patient information anonymized and stored securely in accordance with institutional and regulatory requirements.

Furthermore, given the genetic analysis component of the study, additional ethical considerations were addressed, particularly regarding the implications of genetic findings for patients and their families. Written informed consent was signed by all the parents before it

was performed. Families were informed about the possibility of identifying inherited variants and the potential need for further family screening. Genetic counselling services were made available to assist families in understanding their results and any associated health implications.

IX. STATISTICAL ANALYSIS

Statistical analysis was performed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp, Armonk, NY, USA). A structured database was designed in SPSS to systematically compile all clinical variables, including general patient characteristics, results from initial diagnostic tests, and therapeutic interventions. Additionally, follow-up data were incorporated, with a particular focus on clinical status and CMR findings at 12 months and final clinical status at the end of the study period. A data-cleaning process was conducted to identify missing values, which were supplemented with information retrieved from electronic medical records, where available.

Descriptive statistics were used to summarize the data. Categorical variables were presented as absolute frequencies and percentages, while continuous variables were expressed as medians with interquartile ranges, given the non-normal distribution of the dataset. The Shapiro-Wilk test was used to assess normality.

For comparative analyses, categorical variables were analysed using the Fisher's exact test, while continuous variables were compared using the Mann–Whitney U test. Spearman's rank correlation coefficient was applied to evaluate associations between continuous non-normally distributed variables. A p-value <0.05 was considered statistically significant.

Additional subgroup analyses were performed where applicable to evaluate potential differences in clinical outcomes based on genetic findings, imaging parameters, and therapeutic responses. Statistical significance was adjusted for multiple comparisons using the Bonferroni correction when necessary.

RESULTS:

CORE PUBLICATIONS OF THE THESIS

FIRST ARTICLE

Title of the article:

New-onset heart failure in infants: when the aetiological diagnosis becomes a challenge

Authors:

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SUMMARY OF THE RESULTS

1. Study population and group stratification

This study included infants with new-onset heart failure who underwent both CMR (including tissue mapping) and EMB, and who fulfilled the Marburg immunohistological criteria for inflammatory cardiomyopathy. None of the patients had a family history of dilated cardiomyopathy. Despite indistinguishable clinical presentations at onset, disease progression and the identification of pathogenic or likely pathogenic variants in cardiomyopathy-associated genes enabled the classification of patients into two groups: those with presumed virus-mediated myocarditis (negative genetic testing) and those with genetically mediated DCM.

2. Clinical evolution and prognostic differences

Although initial presentation was clinically indistinguishable between groups, infants without genetic variants were often associated with PVB19 detection in myocardial tissue and exhibited favourable outcomes with full recovery of cardiac function. Conversely, infants harbouring P/LP variants in cardiomyopathy genes showed worse clinical evolution, including persistent ventricular dysfunction and, in some cases, progression to heart transplantation.

3. Histopathological findings on EMB

While all patients met the Marburg immunohistological diagnostic criteria for myocarditis, EMB findings revealed significantly greater inflammatory cell infiltration and oedema in patients without genetic variants (those with better outcomes) compared to the genetically confirmed DCM group. The extent of T-lymphocyte infiltration correlated strongly with troponin levels, supporting its clinical relevance. In contrast, necrosis and fibrosis levels did not differ significantly between the two groups.

4. CMR findings and diagnostic performance

CMR parameters, specifically those defined by the 2018 Lake Louise Criteria, were effective in detecting myocardial inflammation but did not allow reliable discrimination between virus-mediated and genetically mediated forms in this infant population. Late gadolinium enhancement was the only imaging feature associated with virus-mediated myocarditis, ($p = 0.016$), suggesting its potential utility as a supportive diagnostic clue in these infants. However, tissue mapping values (native T1, T2, and ECV) did not differ between groups, and no correlation was found between CMR findings and histological inflammation.

5. Clinical implications and diagnostic strategy

In infants presenting with heart failure and histologically confirmed myocardial inflammation, CMR and EMB alone are insufficient to determine the underlying aetiology. This study underscores the need for an integrative diagnostic approach that combines histopathology (not only fulfilment of Marburg criteria but also quantification of inflammation and oedema), CMR (particularly LGE patterns), troponin levels, viral genome detection, and systematic genetic testing. Accurate aetiological classification is essential to guide targeted treatment strategies and inform prognosis.

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BRIEF REPORT

New-onset heart failure in infants: when the aetiological diagnosis becomes a challenge

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Abstract

This study aimed to report the findings of cardiac magnetic resonance imaging (CMR) with quantitative mappings in infants presenting with new-onset heart failure, as well as to assess the capabilities of endomyocardial biopsy (EMB) and CMR in detecting inflammatory cardiomyopathies and determining their etiology. In a prospective analysis of infants who underwent CMR with tissue mappings, EMB, and genetic testing, the sample was categorized into two groups: those with inflammatory cardiomyopathy and negative genetics (indicative of possible myocarditis) and those with positive genetics (indicative of possible dilated cardiomyopathy). All patients exhibited similar clinical presentations, echocardiographic dysfunction, and elevated troponins and NT-proBNP levels. Additionally, they all met the diagnostic criteria for inflammatory cardiomyopathy based on EMB findings (≥14 mononuclear cells, ≥7 T-lymphocytes/mm2). EMB results unveiled significant differences in the presence of inflammation and edema between the two groups, with higher troponin levels correlating with increased inflammation. Notably, when focusing on CMR, neither the classic criteria nor the 2018 Lake Louise criteria (LLC) could effectively differentiate between the two groups. Only late gadolinium enhancement (LGE) appeared to be associated with myocarditis in this cohort, while other LLC and tissue mappings did not exhibit a similar correlation. Importantly, there was no observed correlation between the inflammation detected through EMB and CMR.

Conclusions: The onset of heart dysfunction in infants can result from either inherited factors or viral infections, both of which may involve inflammation. However, the precise role of EMB and CMR in determining the etiology of such cases remains poorly defined. While CMR demonstrates high sensitivity in detecting inflammation, our experience suggests that it may not effectively differentiate between these two groups. A comprehensive diagnostic approach is essential when addressing this challenge, which includes considering EMB (with attention to the number of T-lymphocytes and the presence of oedema), specific CMR criteria, notably LGE and tissue mappings, as well as the identification of viral agents in cardiac tissue and troponin levels. Additionally, genetic tests should be conducted when evaluating these patients.

What is Known:
- *EMB is the gold standard diagnostic test for myocarditis but it is not universally accepted.*
- *The diagnostic value of the 2018-LLC in pediatric patients is still undefined.*

What is New:
- *Both EMB and CMR may show inflammation in infants with new-onset heart failure of any aetiology.*
- *A global approach should be used when facing this diagnostic challenge, including the EMB (number of T-lymphocytes and oedema), some CMR criteria, specially LGE and mappings, the detection of viral agents in cardiac tissue and troponins. Genetic tests should also be performed when studying these patients.*

Keywords Inflammatory cardiomyopathy · Myocarditis · Dilated cardiomyopathy · Endomyocardial biopsy · Cardiac magnetic resonance · Heart failure

Abbreviations

CMR	Cardiac magnetic resonance imaging
DCM	Dilated cardiomyopathy
ECV	Extracellular volume
EGE	Early gadolinium enhancement

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Extended author information available on the last page of the article

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EMB	Endomyocardial biopsy
IQR	Interquartile range
LGE	Late gadolinium enhancement
LLC	Lake Louise criteria
LV	Left ventricle
LVEF	Left ventricle ejection fraction
PCR	Polymerase chain reaction
PVB19	Parvovirus B19

Introduction

The aetiological diagnosis of infants presenting with new-onset heart failure may be challenging [1]. Acute myocarditis is usually the first clinical suspicion, but it can also be the debut of a genetic dilated cardiomyopathy (DCM).

Endomyocardial biopsy (EMB) is the *gold standard* for diagnosing myocarditis, however, it's not widely used in children as it's invasive and may provoke severe complications [2, 3]; also due to the increasing reliability on cardiac magnetic resonance imaging (CMR) and clinical and laboratory data [1].

The classic Lake Louise criteria (LLC) for CMR (T2-weighted images, early gadolinium enhancement (EGE) and late gadolinium enhancement (LGE)) [4] were initially used to diagnose myocarditis, despite its low sensitivity in patients with heart failure [5]. In 2018, implementing quantitative mapping techniques led to a revision of the original LLC [6, 7]. The 2018-LLC include T1-based imaging (native T1 relaxation times, extracellular volume (ECV) or LGE) and T2-based (T2 relaxation times or T2-weighted intensity) [6, 8, 9]. To date, the diagnostic value of the 2018-LLC in paediatric patients is not fully defined [8].

This study aimed to describe the findings of CMR with quantitative mappings in infants with new-onset heart failure; also, the ability of EMB and CMR to detect inflammatory cardiomyopathies in infants and to determinate its aetiology.

Methods

This prospective study included infants admitted with new-onset heart failure from December 2017 to December 2022 that underwent a CMR with tissue mappings and an EMB.

CMR was performed with a 1.5 T Magnetom Avanto (Siemens Medical System) with cardiac synchronization. White blood sequences (SSFP), T2 (T2W-STIR) and T1-weighted sequences (TSE) before and after the administration of gadolinium (0.2 mmol/kg) and delayed uptake of contrast (PSIR-SSFP) were performed. Tissue mappings [6, 8, 9] were measured on the posterior wall of the left ventricle (LV) and the interventricular septum in end-diastole in short-axis views. Normal values for healthy children at our centre were: native T1 (mean 1013 ms, SD 40.3), ECV (25.6%, SD 3.8), T2 (48.6 ms, SD 4.2).

EMB was executed in patients ≥ 6 months and ≥ 8 kg [2] with new-onset ventricular dysfunction of unknown aetiology, presenting with LV ejection fraction (LVEF) < 35% and either needed extracorporeal membrane oxygenation or had haemodynamic compromise without improvement after ≥ 2 weeks of treatment. In patients < 6 months or < 8 kg, the decision to perform an EMB was individualized. Samples obtained from the interventricular septum underwent Haematoxylin–eosin, Mason's trichrome and immunohistochemical stains for CD45, CD20, CD3, and CD68. Viral PCR (polymerase chain reaction) were also performed. Inflammatory cardiomyopathy was determined following the Marburg immunohistological criteria: ≥ 14 mononuclear cells with ≥ 7 T-lymphocytes per mm2 [3].

Genetic testing was performed in all patients, using the TruSight Cardio Panel (Next Generation Sequencing technology), including exonic and flanking intronic regions of 174 genes related to cardiovascular diseases. The identification of pathogenic or likely-pathogenic mutations was used to divide the sample into two groups: inflammatory cardiomyopathy with positive genetics (possible DCM) vs negative genetics (possible myocarditis).

Statistical analysis was performed using SPSS 25.0 (IBM). Nominal data were described using proportions and continuous quantitative data employing medians and interquartile range (IQR) as the sample did not present a normal distribution. Continuous variables were assessed using Mann–Whitney U test and correlations where determined using Spearman's Rank. P values < 0.05 were considered statistically significant.

Results

10 infants of median age 0.93 years [IQR 0.5–2.4] were included. In Table 1 we summarize the results of their CMR and EMB; all fulfilled the immunohistological diagnostic criteria for inflammatory cardiomyopathy [3]. Pathogenic or likely-pathogenic mutations were detected in 6 out of 10 patients.

Echocardiographic assessment at diagnosis showed severe dysfunction (median LVEF 24%, [IQR 17.75–33]), dilated LV (z-score 6.88 [3.98–8.5]) and left atrium, with indirect signs of high left atrial pressure (mitral E/e' ratio of 18 [15.83–23.23]). CMR confirmed the results: LV dysfunction (LVEF 20%, [15–32.75]) and dilation (LV end-diastolic volume 169.5 ml/m2 [113.5–213.5]). Analytically, elevated high-sensitivity troponins (106 ng/L [55.4–215.88]) and N-terminal-prohormone BNP (25902U/L, [16612.5–48194.5]) were detected. No

Table 1 Qualitative and quantitative cardiac magnetic resonance parameters, endomyocardial biopsy, genetic tests and follow-up

Patient	Age	Genetic test	Classic Lake Louise criteria			Quantitative mappings			Endomyocardial biopsy					Evolution		
			T2 signal intensity ratio	EGE	LGE	Native T1 (msec)	ECV (%)	T2 (msec)	Viral PCR	T cells / mm_2	Necrosis	Fibrosis	Edema	LVEF at debut (%)	Need for ECMO	Follow-up
1	3 years	Neg	Neg	Neg	Positive	*IVS:* 1078 *LVPW*: 1080	32.38 31.02	57 56	PVB19	180	Yes	No	Yes	30	No	Recovered
2	9 months	Neg	Positive	Positive	Positive	*IVS*: 1247 *LVPW*: 1251	44.56 45.27	64 58	PVB19	70	Yes	No	Yes	13	No	Recovered
3	6 months	Neg	Positive	Positive	Positive	*IVS*: 1191 *LVPW*: 1159	35.70 31.95	66 63	Neg	50	No	No	Yes	15	No	Recovered
4	21 months	Neg	Positive	Neg	Positive	*IVS*: 1139 *LVPW*: 1178	46.57 55.77	54 59	PVB19	15	No	Yes	Yes	43	Yes	Recovered
5	2 months	RPL3L c.922G > A c.970G > A	Positive	Positive	Neg	*IVS*: 1226 *LVPW*: 1230	36.7 29.59	67 72	Neg	23	No	No	Yes	32	Yes	Heart transplant
6	6 months	FLNC c.510delA	Neg	Neg	Neg	*IVS*: 1179 *LVPW*: 1115	39.00 33.72	58 57	Neg	9	No	No	No	15	Yes	Heart transplant
7	2 years	MYH7 c.2710C > T	Positive	Neg	Positive	*IVS*: 1086 *LVPW*: 1022	30.63 35.03	70 65	Neg	14	Yes	No	No	35	No	Heart transplant
8	11 months	SDHA c.1660C > T c.1549A > G	Positive	Neg	Neg	*IVS*: 1242 *LVPW*: 1195	36.00 38.00	75 69	Neg	18	Yes	Yes	No	15	Yes	Persistent cardiac dysfunction
9	2 years	MYH7 c.4013A > C TNNI3 c.292C > T	Positive	Neg	Positive	*IVS*: 1175 *LVPW*: 1155	38.37 34.67	58 56	Neg	15	No	No	No	15	No	Persistent cardiac dysfunction
10	10 months	TNNI3 c.204delG	Positive	Positive	Neg	*IVS*: 1209 *LVPW*: 1267	30.36 32.64	66 62	PVB19	11	No	No	No	25	Yes	Heart transplant

DCM Dilated cardiomyopathy, *ECMO* Extracorporeal membrane oxygenation, *ECV* Extracellular volume fraction, *EGE* Early gadolinium enhancement, *IVS* Interventricular septum, *LGE* Late gadolinium enhancement, *LVEF* Left ventricle ejection fraction, *LVPW* Left ventricle posterior wall, *Neg* Negative, *PCR* Polymerase chain reaction, *PVB19* Parvovirus B19

statistically significant differences on these tests were observed between groups.

Patients with inflammatory cardiomyopathy and negative genetics manifested progressive cardiac function recovery. In 3/4 patients, positive PCR for Parvovirus B19 (PVB19) was revealed in cardiac tissue. Contrarily, the patients with positive genetics presented poor outcomes (persistent cardiac dysfunction or heart transplantation); in this group PVB19 was detected in 1/6 patients.

EMB, performed a median of 6.5 days [2.5–13.25] after the CMR, revealed significant differences in inflammation ($p=0.038$) between patients with negative (60 T-lymphocytes/mm2 [23.75–152]) and positive genetics (14.5 [10.5–9.25]). Higher levels of troponin were associated with greater inflammation on EMB (Spearman Rank Correlation 0.695, $p=0.026$). Besides, oedema was also more frequent in patients with negative genetics (100% vs 16.67%, $p<0.005$). No difference was found on the presence of necrosis (2/4 vs 2/6, $p=0.599$).

Focusing on CMR, classic LLC may not differentiate between groups in this sample (3/4 vs 4/6, $p=0.777$). The 2018-LLC may not either, because all the patients except for one (patient 6), fulfilled the criteria. When analyzing each criteria individually, LGE may be associated with negative genetics (possible myocarditis) (4/4 vs 2/6, $p=0.016$), being the most sensitive (100%) and specific (66.7%) parameter; the other parameters may not: T2-weighted (3/4 vs 5/6, $p=0.749$), EGE (2/4 vs 2/6, $p=0.599$). Inflammation highlighted through mappings may not either: T1 mapping (1184.5 [1104.5–1236] vs 1204.5 [1152.75–1248.25], $p=0.762$), ECV (40.48 [33.21–53.15] vs 37.34 [32.14–38.52], $p=0.610$), T2 mapping (61.5 [57.5–65.5] vs 68 [58–72.75], $p=0.171$). Moreover, a patchy affectation on CMR, quantified as the difference in mappings between the LV posterior wall and the interventricular septum, would not be decisive either: T1 mapping ($p=0.114$), ECV ($p=0.610$), T2 mapping ($p=1$).

Eventually, no correlation was observed between inflammation detected in EMB and positive LLC ($p=1$), nor with quantitative mappings analized in the septum: Spearman Rank Correlation with T1 mapping 0.146 ($p=0.688$), ECV 0.061 ($p=0.868$) and T2 mapping -0.070 ($p=0.847$).

Discussion

This study presents the clinical scenario of very young patients with serious cardiac dysfunction being, in this life-threatening situation, very important to refine diagnosis and prognosis. To date, there are no large reports comparing EMB and CMR in infants.

All our patients had a similar clinical presentation and all meet the diagnostic criteria for inflammatory cardiomyopathy on EMB [3]. However, 6 patients had pathogenic or likely-pathogenic mutations and presented poor outcomes, and 4 had negative genetic studies and recovered cardiac function.

A genetic predisposition has been described in myocarditis [3], but on the other hand, inflammation on EMB is not specific for myocarditis and may also be detected in infants with heart failure of any aetiology, as it can be triggered by heart failure itself independently of any active infection [10]. Furthermore, recent research in adult patients has introduced the "Double-Hit Theory" for the pathogenesis of myocarditis. According to this theory, an inflammatory mechanism might represent a second 'hit' driving the onset of heart affection in cardiomyopathies but it could only represent a 'hot phase' in the natural history of these conditions. Additionally, recent evidence highlights the vulnerability to acute myocarditis in genetically predisposed individuals [11]. In our series, the absence of pathogenic mutations was associated with EMB changes (increased inflammation, oedema and detection of viral agents) and to a complete recovery; the detection of any likely-pathogenic mutation predicted poor outcomes.

Ideally, EMB data should be combined with CMR and clinical information to refine the diagnosis. In contrast to recent literature [1], higher troponin levels may be associated with greater inflammation detected by EMB. Viral PCR positivity in cardiac tissue and complete echocardiographic recovery would indicate myocarditis albeit PVB19 genome has been found in healthy-people's myocardium, suggesting that this may not always imply an active infection [12], like patient 10.

CMR allows non-invasive myocardial inflammation assessment. A higher diagnostic performance after the implementation of mapping techniques has been established [7, 9, 13]; nevertheless, experience in children is limited [1, 8]. Our study confirms its capacity to detect inflammation, but not to reach the aetiological diagnosis, since we found inflammation in both groups [10]. A patchier affection might not be decisive either.

Regarding each parameter individually, LGE seems a sensitive parameter. Evidence suggests that it would increase the diagnostic capacity of mappings to detect myocarditis [7]. EGE, however, might be removed from the CMR protocol without changing the diagnostic accuracy [13]. Quantitative mappings may be useful, but the small sample size does not allow establishing predictions. In adult literature, mappings are the best-performing parameters for diagnosing myocarditis [6, 7] but differential diagnosis is usually with coronary ischemia and noninflammatory cardiomyopathies [1]. Particularly, T2-mappings can help to discriminate between acute and subacute inflammation as they are insensitive to fibrosis [8, 9]. Further research is needed to investigate the optimal parameter combination for children.

Greater inflammation on EMB was not associated with CMR parameters in this study. Time interval between tests could bias inflammation quantification. Patchy inflammation in the myocardium may also be a cause; besides, myocarditis occurs predominantly in the free wall of LV, inaccessible with EMB techniques [1, 2]. A high correlation has been described in adults with infarct-like presentations [5, 13] but not in infants with heart failure.

Tu summarize, new-onset heart dysfunction in infants may be inherited, secondary to viral infections or even have a combined etiology [3, 11] and inflammation can be found in all [10]. The role of EMB and CMR in determining the aetiology of heart failure in infants remains poorly defined [1, 2, 8]. CMR is very sensitive in detecting inflammation but, in our experience, it may not differentiate between groups. A global approach should be used when facing this diagnostic challenge, including the EMB (number of T-lymphocytes and oedema), some CMR criteria, specially LGE and mappings, the detection of viral agents in cardiac tissue and troponins. Genetic tests should also be performed when studying these patients.

The main limitation of this study is the small sample. Heart failure is infrequent in infants, and is not always safe to perform an EMB. This makes it very difficult to recruit patients and to consequently get significant results. Furthermore, negative genetic studies would not exclude a genetic cause as we may have missed likely-pathogenic variants in genes not included in the panel. Larger multicentre studies would help to achieve stronger conclusions.

Authors' contributions All authors contributed to the conception and design of the work. Lucía Riaza, Paola Dolader, Anna Sabaté-Rotés and Ferran Rosés-Noguer participated in data collection. Analysis and interpretation were performed by Roger Esmel-Vilomara and Ferran Gran. The first draft was written by Roger Esmel-Vilomara. The draft was read and approved by all the authors.

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Data availability The data supporting the findings of this study are available upon request. Please contact the corresponding author for access to the dataset or any additional information related to the data.

Declarations

Ethics approval This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Vall d'Hebron Hospital Campus.

Consent to participate Not applicable.

Consent for publication Informed consent for publication was obtained.

Competing interests The authors declare no competing interests.

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SECOND ARTICLE

Title of the article:

High specificity ECG patterns for Parvovirus B19 myocarditis in children: Bridging ECG findings to etiological diagnosis.

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SUMMARY OF THE RESULTS

1. Identification of a distinctive ECG pattern

This study identified a highly specific electrocardiographic pattern in the limb leads of paediatric patients with PVB19 myocarditis. The pattern, characterised by peaked P waves, low QRS voltages, and repolarization abnormalities (manifesting as flattened or inverted T waves, with or without QTc prolongation), was present in 70% of patients with PCR-confirmed

PVB19 myocarditis. In contrast, only a minority of patients with myocarditis due to other viral pathogens exhibited the complete pattern. This combination demonstrated a specificity of 98% and sensitivity of 70%, suggesting its potential as a valuable early marker for PVB19 as the underlying aetiology in paediatric myocarditis.

The proposed ECG pattern likely reflects underlying myocardial inflammation. Peaked P waves may indicate increased atrial pressure secondary to a restrictive filling pattern and diastolic dysfunction caused by myocardial oedema. Low QRS voltages are also commonly linked to myocardial oedema, reflecting the dampering of electrical signal amplitude. Repolarization abnormalities may result from regional inflammation affecting subepicardial or transmural conduction.

2. ECG abnormalities in PVB19 myocarditis

All patients with PVB19 myocarditis presented with abnormal ECG findings. Repolarization abnormalities were observed in 100% of PVB19 cases, compared to 35.1% in those with other viral causes. Low QRS voltages were identified in 80% of PVB19 patients, significantly higher than the 17.5% observed in the control group. Similarly, peaked P waves were present in 76.7% of PVB19 cases, while only 8.8% of patients with non-PVB19 myocarditis exhibited this feature. Conversely, ECG findings such as Q waves, wide QRS complexes, and ST-segment elevationswere more prevalent in myocarditis caused by other viruses.

3. Potential clinical implications

Early recognition of this ECG pattern may assist clinicians in suspecting PVB19 myocarditis during the initial evaluation, potentially prompting earlier microbiological testing and timely initiation of pathogen-targeted or supportive therapies. This is particularly relevant in clinical settings where access to advanced diagnostic modalities such as endomyocardial biopsy (EMB) or cardiac magnetic resonance (CMR) is limited. While ECG alone cannot confirm the viral aetiology, this distinctive pattern offers a useful, non-invasive diagnostic clue that can support clinical decision-making and help streamline patient management.

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Original Article

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High specificity electrocardiogram patterns for parvovirus B19 myocarditis in children: bridging electrocardiogram findings to aetiological diagnosis

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Abstract

Introduction: Parvovirus B19 (PVB19) myocarditis is a life-threatening condition with high morbidity and mortality in children. While electrocardiograms are commonly used in the early assessment of myocarditis, no specific electrocardiogram pattern has been consistently linked to PVB19. The objective of this study is to identify a distinctive electrocardiogram pattern associated with PVB19 myocarditis and evaluate its diagnostic accuracy. *Methods:* This retrospective case–control study included 77 paediatric patients diagnosed with acute myocarditis at a single centre in Barcelona over 16 years (August 2008–September 2024). Twenty patients had PVB19 myocarditis, confirmed by polymerase chain reaction in blood or endomyocardial biopsy, while 57 patients had myocarditis caused by other viruses. Electrocardiogram were assessed by three cardiologists blinded to the aetiological diagnosis. *Results:* A specific electrocardiogram pattern in the limb leads, characterised by peaked P waves, low QRS complex voltages, and altered repolarisation (manifesting as negative or flat T waves, with or without QTc prolongation), was observed in 14 of 20 patients (70%) with PVB19 myocarditis. Two additional patients exhibited low voltages and altered repolarisation without peaked P waves, and all demonstrated repolarisation abnormalities. In contrast, only 1 of 57 patients with myocarditis from other viruses exhibited the full electrocardiogram pattern. The pattern demonstrated a specificity of 98% and a sensitivity of 70% for PVB19 myocarditis. *Conclusion:* The identified electrocardiogram pattern shows strong diagnostic specificity for PVB19 myocarditis in paediatric patients and may serve as a useful early diagnostic tool. Further multicentre studies are needed to confirm these findings and explore their clinical implications.

Introduction

Myocarditis is an inflammatory disease of the heart primarily caused by viral infections .[1,2] Its incidence is estimated at 10 to 20 cases per 100,000 individuals, and it represents a significant cause of morbidity and mortality in children.[2–4] Among the responsible viral agents, Parvovirus B19 (PVB19) has emerged as the leading cause in the paediatric population.[4–6] Nonetheless, a limited number of published series focuses specifically on PVB19 myocarditis in children.[5,7,8]

The clinical presentation of myocarditis varies widely, from asymptomatic forms to life-threatening conditions such as cardiogenic shock or sudden cardiac death.[1–3] This nonspecific clinical presentation increases the risk of misdiagnosis or delays in diagnosis, which can delay the initiation of treatment and negatively impact survival chances.[4,9]

The electrocardiogram is a readily available, low-cost diagnostic tool frequently utilised initial assessment of suspected myocarditis.[1,3,9,10] Although electrocardiogram abnormalities are found in nearly all children with myocarditis, no distinctive electrocardiographic changes can definitively diagnose the condition, and the test has limited sensitivity and specificity.[9–11]

This study aims to evaluate the electrocardiogram changes in paediatric patients with PVB19 myocarditis and determine whether a specific electrocardiogram pattern is associated with PVB19.

Methods

We performed a retrospective case control study including 77 consecutive paediatric patients diagnosed with acute myocarditis in a single centre in Barcelona over 16 years (August 2008 to September 2024). Inclusion criteria required a diagnostic confirmation by endomyocardial

biopsy or cardiac MRI, with the myocarditis viral aetiology confirmed by polymerase chain reaction testing on endomyocardial biopsy or blood samples. Twenty patients were attributed to PVB19 (Group A), whereas the other 57 were caused by other viruses (Group B).

Demographics, initial presentation, severity of illness, aetiology, echocardiographic assessment, and electrocardiogram analysis were studied for both groups. Standard 12-lead electrocardiograms upon admission were evaluated by three cardiologists who were blinded to the aetiological diagnosis and Group A and B electrocardiogram were compared.

Statistical analysis was conducted using SPSS for Windows version 25.0 (Armonk, NY, USA: IBM Corp.). Categorical data were described using proportions, while quantitative continuous data were expressed as medians with interquartile ranges, given the non-normal distribution of the sample. Comparisons were made using Fisher's exact test for categorical data and the Mann–Whitney *U* test for continuous variables.

Ethical approval for this study was granted by the local ethics committee, ensuring compliance with ethical standards and the protection of participants' rights and safety. All activities conducted in this research adhered to the ethical principles outlined in the 1975 Declaration of Helsinki. Informed consent for publication was obtained from the parents of each patient.

Results

A total of 20 episodes of myocarditis involving 19 patients were identified. The median age was 19 months, and 13 (65%) were male. All patients presented with complicated myocarditis, manifesting either as heart failure, fulminant myocarditis or ventricular tachycardia, and requiring mechanical circulatory support with extracorporeal membrane oxygenation in 7 patients (35%). Detailed epidemiological and clinical data are shown in Table 1.

All patients exhibited abnormal electrocardiogram findings at presentation. Electrocardiogram abnormalities are detailed in Table 1. A specific pattern in the limb leads, characterised by peaked P waves, low QRS voltages and altered repolarisation (manifesting as negative or flat T waves with or without QTc prolongation), was observed in 14 patients (14/20, 70%). Notably, all patients had altered repolarisation (100%). In addition to the 14 patients with the complete pattern, two additional patients showed low voltages and altered repolarisation without peaked P waves.

Figure 1 provides examples of the described electrocardiogram pattern. The presence of more severe myocardial dysfunction ($p = 0.323$) or pericardial effusion ($p = 0.521$) was not associated with the described electrocardiogram pattern or any specific electrocardiogram finding independently.

During the same period, 57 patients with myocarditis not attributable to PVB19 were included, of whom 35 (35/57, 61.4%) presented with complicated myocarditis, including cardiogenic shock or heart failure. Among these patients, only one exhibited the complete electrocardiogram pattern described in the limb leads (1/57, 1.8%). Twenty patients presented an abnormal repolarisation (20/57, 35.1%), ten exhibited low voltages (10/57, 17.5%), and five had peaked P waves (8.8%).

These findings suggest that the described electrocardiogram pattern is significantly associated with PVB19 myocarditis ($p < 0.001$), showing a specificity of 98% and a sensitivity of 70% for identifying PVB19 in patients with myocarditis.

Discussion

Electrocardiogram is universally recommended as a first-line investigation for patients with suspected myocarditis.[1,2] Numerous retrospective studies have demonstrated that electrocardiogram abnormalities are detected in nearly all patients,[9,11] although some studies report a subset of patients with normal electrocardiogram findings.[10] However, the potential association between specific electrocardiogram patterns and a particular causative agent has not yet been studied, particularly in paediatric populations where research is limited, and sample sizes are smaller. This study is the first to present an electrocardiogram pattern that could potentially help identify PVB19 as the underlying cause of myocarditis in paediatric patients.

Previous studies examining electrocardiogram alterations during acute myocarditis have identified sinus tachycardia and repolarisation abnormalities, particularly T-wave inversion, as the most common electrocardiogram findings.[9,11] In our study, repolarisation abnormalities were observed in all patients with PVB19 myocarditis, compared to 35.1% of patients with myocarditis caused by other virus. Repolarisation abnormalities have been reported in patients with myocarditis of varying severity, from non-complicated to fulminant myocarditis, with a prevalence ranging from 9 to 48%.[9,11,12]

The pathogenic link between myocardial oedema and repolarisation abnormalities remains to be fully elucidated. However, it has been proposed that regional or transmural myocyte repolarisation inhomogeneity secondary to the inflammatory process could be responsible.[9,12] In this context, T-wave inversion may result from inflammatory or fibrotic infiltration beginning in the subepicardium, where depolarisation and repolarisation become more delayed compared to the subendocardium, leading to a transmural repolarisation gradient.[13] In line with this hypothesis, T-wave inversion has been independently associated with the extent of myocardial necrosis and oedema detected by MRI, with transmural oedema distribution strongly correlated with the location of T-wave inversion.[12]

Low QRS voltages were observed in 16 of our patients (16/20, 80%), a finding traditionally associated with pericardial effusion or extracardiac conditions such as obesity or emphysema.[13] However, low QRS voltages have also been documented in myocarditis independent of pericardial effusion,[11] a finding confirmed by this study. The proposed mechanisms for low QRS voltages in this setting include expansion of extracellular spaces by oedema and fibrosis in the ventricular wall. Pulmonary or peripheral oedema can also contribute to reducing voltage amplitude by increasing impedance to electrical conduction.[9] In cardiomyopathies, low QRS voltages are observed in 3–6% of patients with dilated cardiomyopathy and in up to 41% of patients with certain forms of non-dilated left ventricular cardiomyopathy.[13] The markedly higher prevalence of low voltages in PVB19 myocarditis in our cohort (80%) highlights the relevance of this electrocardiogram finding in this specific aetiology.

Additionally, peaked p waves on electrocardiograms, which indicate increased atrial pressures, are often a consequence of heart failure. Concurrently, myocardial oedema can further diminish ventricular compliance, exacerbating atrial pressure by creating a restrictive filling pattern and diastolic dysfunction. The impaired ventricular contraction and relaxation can also lead to atrioventricular valve regurgitation, further exacerbating atrial volume overload and pressure elevation.[13]

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Table 1. Demographic inf ormation, clinical presentation, and electrocardiographic abnormalities upon presentation. Categorical data is presented as *n* (%) and continuous variables as median and interquartile ranges (IQR)

Clinical presentation	Group A (PVB19) n =20 episodes	Group B (other viruses) *n* =57 episodes
Demographics		
Male	13 (65%)	32 (56.1%)
Age in months at presentation (median, IQR)	19 (16.25)	99 (148)
Initial clinical presentation		
Cardiogenic shock	10 (50%)	23 (40.4%)
Heart failure	9 (45%)	10 (17.5%)
Chest pain	0 (0%)	22 (38.6%)
Arrhythmias	1 (5%)	2 (3.5%)
Severity of illness		
Intensive care unit admission	20 (100%)	40 (70.1%)
Mechanical ventilation	15 (75%)	28 (49%)
Inotropic agents	20 (100%)	35 (61.4%)
Mechanical circulatory assist devices	7 (35%)	11 (19.3%)
Echocardiographic assessment		
Left ventricular ejection fraction (median, IQR)	25 (7)	35 (35)
Left ventricular dilatation	20 (100%)	26 (45.6)
Left ventricle hypertrophy	10 (50%)	15 (26.3%)
Left atrium dilatation	20 (100%)	24 (42.1%)
Mitral regurgitation (moderate to severe)	14 (70%)	18 (31.6%)
Right ventricular dysfunction	6 (30%)	15 (26.3%)
Pericardial effusion	2 (10%)	5 (8.8%)
Microbiological diagnosis (PVB19 PCR)		
Endomyocardial biopsy	16 (80%)	2 (3.5%)
Blood	19 (95%)	14 (24.6%)
Electrocardiographic abnormalities		
Peaked P waves	14 (70%)	5 (8.8%)
Q waves	1 (5%)	10 17.5%)
Low QRS voltages	16 (80%)	10 (17.5%)
Wide QRS complex	2 (10%)	8 (14.0%)
ST elevation	2 (10%)	25 (43.9%)
Altered repolarisation	20 (100%)	20 (35.1)

PVB19 PCR, parvovirus B19 polymerase chain reaction.

Although electrocardiogram is widely used for the early screening of myocarditis,[1,3,10] its sensitivity and specificity for diagnosing myocarditis remain low.[2,9,10] However, once myocarditis is suspected, the presence of this specific electrocardiogram pattern in the limb leads (characterised by peaked p waves, low QRS voltages and abnormal repolarisation) should prompt consideration of PVB19 as the likely cause, with a specificity of 98%. The sensitivity of this pattern, while lower at 70%, is still noteworthy. These findings could have significant clinical implications, not only guiding the selection of appropriate diagnostic tests but also influencing therapeutic decisions.

In particular, recent studies have proposed that immunosuppressive treatment, potentially combined with interferon-beta to suppress active PVB19 transcription in the myocardium, might offer an effective strategy for managing PVB19 myocarditis.[4,5,14] However, in the absence of definitive evidence, targeted immunosuppressive or antiviral therapy should not be initiated based solely on electrocardiogram findings without microbiological confirmation. While early empirical immunomodulatory treatment with corticosteroids may be considered in critically ill patients with cardiogenic shock or severe systolic dysfunction, particularly if this characteristic electrocardiogram pattern is present, this approach remains untested in clinical trials.[2,14]

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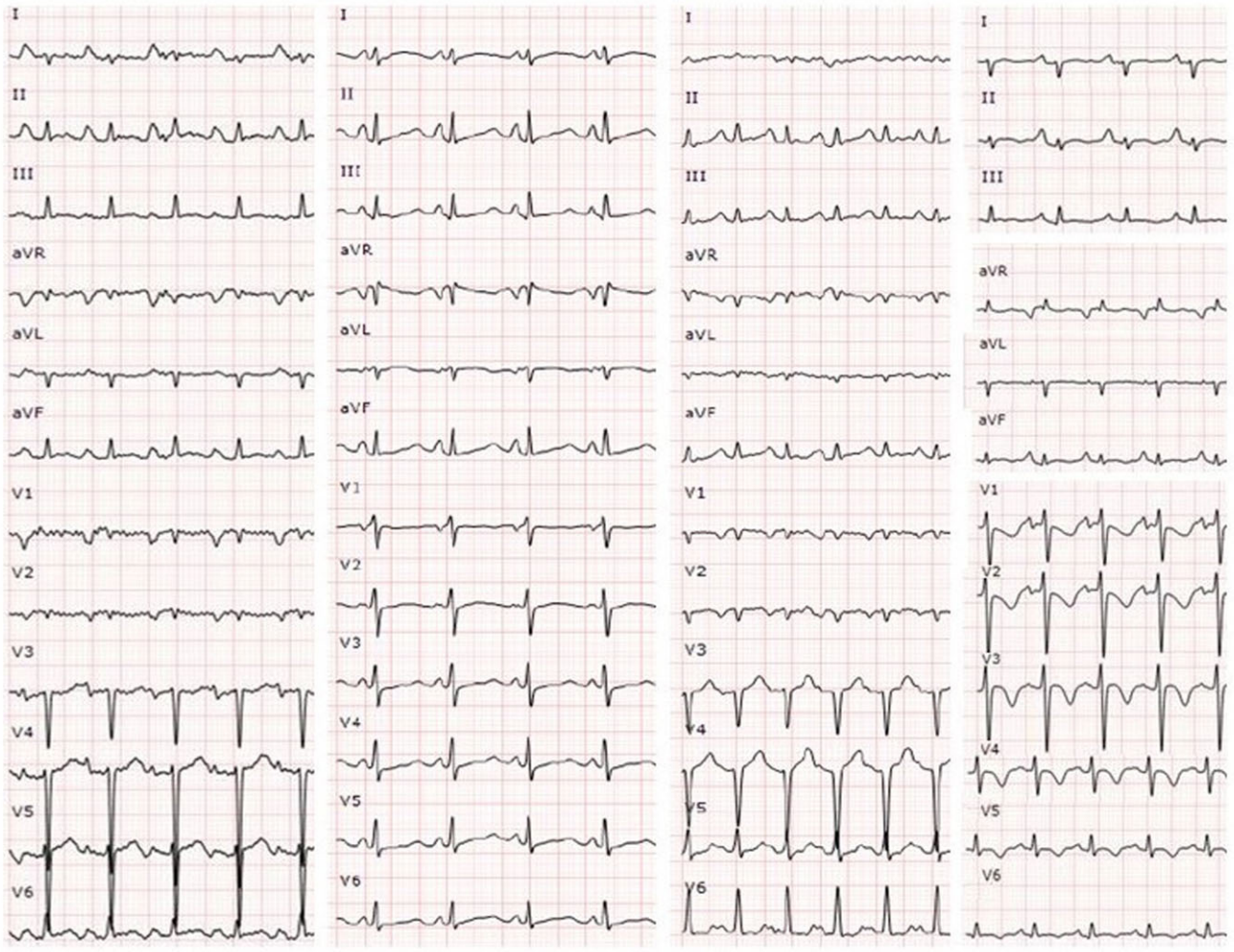

Figure 1. Examples of the PVB19 ECG pattern presented in the limb leads: peaked p waves, low QRS voltages, and repolarisation abnormalities (manifested as negative or flat T waves).

Current guidelines do not provide specific recommendations for empiric immunosuppression in acute myocarditis beyond standard heart failure management.[1,3]

Beyond specialised centres where endomyocardial biopsy is routinely available, this electrocardiogram pattern could serve as a practical and widely accessible tool to aid in the early identification and risk stratification of paediatric myocarditis, particularly in resource-limited settings where advanced diagnostic tools as endomyocardial biopsy or cardiac MRI are not readily available. If validated in larger multicentre studies, its application could contribute to earlier clinical suspicion, optimised diagnostic workflows, and potentially improved patient outcomes.

The primary limitation of this study is its single-centre design, which may impact the generalisability of the findings. Additionally, the small sample size of 20 patients with PVB19 myocarditis, due to the rarity of the disease, restricts the robustness of the conclusions. To validate these results and enhance clinical applicability, future prospective multicentre studies with larger, well-characterised cohorts are needed. These studies should aim to confirm the specificity and sensitivity of this electrocardiogram pattern and assess its predictive value in clinical decision-making. Furthermore, expanding the number of centres and patients would facilitate an evaluation of interobserver agreement, ensuring consistent electrocardiogram interpretation in routine practice.

Conclusion

This study identifies a specific electrocardiogram pattern associated with PVB19 myocarditis in paediatric patients, characterised by peaked P waves, low QRS voltages, and abnormal repolarisation. This pattern demonstrates a high specificity (98%) for PVB19 myocarditis and a sensitivity of 70%. These findings can help in distinguishing PVB19 myocarditis from other aetiologies from the first medical evaluation, guiding clinicians towards appropriate diagnostic and therapeutic strategies. To validate these findings, further research involving larger, multicentric cohorts is essential to confirm the pattern's clinical utility and explore its underlying mechanisms.

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Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national Spanish guidelines on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008, and has been approved by the Vall d'Hebron Hospital Ethics Committee.

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THIRD ARTICLE

Title of the article:

Infarct-like myocarditis in adolescents: Exploring genetic insights from diagnosis through follow-up.

Authors:

Esmel-Vilomara R, Riaza L, Dolader P, Rodríguez-Santiago B, Lasa-Aranzasti A, Muñoz-Cabello P, Fernández-Álvarez P, Figueras-Coll M, Bianco L, Bueno-Gómez A, Vargas-Pons L, Camprubí-Tubella E, Marimon-Blanch C, Sabaté-Rotés A, Rosés-Noguer F, Gran F.

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AWARDED ARTICLE

Recipient of the best presentation award (Manuel Quero Award 2024) at the 15th Congress of the Spanish Society of Paediatric Cardiology (Sociedad Española de Cardiología Pediátrica y Cardiopatías Congénitas, SECPCC)

SUMMARY OF THE RESULTS

1. Prevalence of genetic variants

Pathogenic or likely pathogenic variants in cardiomyopathy-associated genes were identified in 22.2% of adolescents diagnosed with infarct-like myocarditis. Remarkably, none of these patients had a known family history of cardiomyopathy, underscoring that genetic predisposition can remain clinically silent and manifest solely through an initial episode of myocardial inflammation, even in previously healthy children with uncomplicated presentations.

2. Baseline CMR findings and genetic association

All patients met the 2018 Lake Louise Criteria on their initial CMR. While no significant differences were observed in baseline ejection fraction or extent of LGE between genetic-positive and negative patients, the presence of a ring-like LGE pattern and septal involvement were more frequently associated with P/LP variants. These imaging characteristics may represent early markers suggestive of an underlying genetic cardiomyopathy.

3. Follow-up CMR and longitudinal changes

In the CMR follow-up performed at 12-month, patients carrying P/LP variants demonstrated significantly more persistent abnormalities, including a larger extent of LGE ($p = 0.047$) and residual myocardial oedema on T2-STIR sequences ($p = 0.009$). These findings point to ongoing myocardial inflammation and remodelling, supporting the need for long-term imaging surveillance even in cases that initially appear clinically resolved.

4. Myocarditis recurrence and genetic background

Over a median follow-up of three years, 30% of the cohort experienced at least one recurrence of myocarditis. Recurrence was notably more frequent among patients with genetic variants (66.7% vs. 23.8%), although this trend did not reach statistical significance ($p = 0.073$). These recurrent events may represent “hot phases” of an evolving cardiomyopathy in genetically predisposed individuals. This highlights the importance of maintaining both clinical and imaging follow-up even in paediatric patients with apparently mild episodes of myocarditis.

5. Implications for genetic testing

The findings of this study support the consideration of genetic testing in adolescents with infarct-like myocarditis, particularly in the presence of CMR features such as ring-like or septal LGE, or in those with recurrent episodes. Persistent abnormalities and recurrence in gene-positive patients reinforce the paradigm that some cases of myocarditis may reflect the inflammatory onset of inherited cardiomyopathy. Early genetic diagnosis can guide patient management, inform family screening, and design long-term follow-up strategies to improve outcomes.

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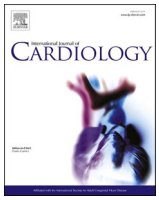

Infarct-like myocarditis in adolescents: Exploring genetic insights from diagnosis through follow-up

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ABSTRACT

Background: Myocarditis has traditionally been considered an acquired condition, but recent evidence suggests a genetic contribution, primarily in complicated cases. Data on pediatric uncomplicated or infarct-like myocarditis remain scarce. This study aimed to assess the prevalence of pathogenic or likely pathogenic (P/LP) variants in adolescents with infarct-like myocarditis and their association with clinical and imaging findings.

Methods: This prospective, multicenter study included 30 adolescents diagnosed with infarct-like myocarditis across five hospitals in Catalunya, Spain (2016–2024). Diagnosis was confirmed using the 2018 Lake Louise Criteria on cardiac magnetic resonance imaging (CMR). Follow-up CMR was performed at 12 months, and genetic testing was conducted using a next-generation sequencing panel targeting 174 genes associated with inherited cardiac diseases.

Results: P/LP variants in cardiomyopathy-associated genes were identified in 22.2 % of patients. Baseline CMR showed no significant differences in ventricular function or LGE extent, but a ring-like LGE pattern was significantly associated with genetic findings ($p = 0.025$), while septal involvement showed a p-value of 0.056. Over a median follow-up of 3 years (IQR 2–7), 9 patients (30 %) experienced recurrent myocarditis, more frequently in genetic-positive patients (66.7 % vs. 23.8 %). At 12 months, genetic-positive patients exhibited a greater LGE burden ($p = 0.047$) and persistent myocardial edema on T2-STIR ($p = 0.009$), suggesting ongoing myocardial remodeling.

Conclusions: The high prevalence of P/LP variants in infarct-like myocarditis highlights the need for genetic testing, particularly in patients with a ring-like LGE pattern or septal involvement. Persistent CMR abnormalities and symptomatic recurrences in genetic-positive cases support long-term monitoring, even in seemingly uncomplicated presentations.

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1. Introduction

Myocarditis, an inflammatory condition of the myocardium, is an uncommon but significant cause of morbidity and mortality in pediatric patients [1,2]. In children, it often manifests with a spectrum of clinical presentations, ranging from mild chest pain to heart failure or even sudden cardiac death [1,3]. Myocarditis can be classified as complicated, involving heart failure, arrhythmias or fulminant presentations, and uncomplicated or infarct-like (acute coronary syndrome-like) myocarditis. The latter are characterized by chest pain, elevated cardiac biomarkers, and ST-segment elevation mimicking acute myocardial infarction, but without significant left ventricular (LV) dysfunction or coronary artery involvement [[3–5]]. The clinical diagnosis is usually confirmed by cardiac magnetic resonance imaging (CMR) which has emerged as the reference tool for the non-invasive diagnosis of myocarditis [6,7].

While myocarditis was traditionally considered an acquired condition, recent evidence highlights the relevance of genetic factors [8–10]. The prevalence of pathogenic or likely pathogenic (P/LP) genetic variants in myocarditis varies widely across studies [11–13], ranging from 4 % in uncomplicated cases to over 20 % in complicated myocarditis [10]. However, most available data focus on adult populations and complicated presentations, such as fulminant myocarditis, heart failure, or arrhythmogenic presentations [8–10]. Limited studies have explored the genetic background in pediatric patients [12–14] and even fewer have focused on uncomplicated or infarct-like presentations, where the clinical course often seems benign and genetic predisposition may be underrecognized.

Emerging evidence supports the connection between genetics and myocarditis pathogenesis. The “second hit” hypothesis suggests that viral infections or other environmental triggers may precipitate myocarditis in genetically predisposed individuals, initiating an inflammatory cascade [15,16]. Episodes of myocarditis may also represent hot phases of cardiomyopathies, particularly dilated cardiomyopathy (DCM), non-dilated LV cardiomyopathy (NDLVC) and arrhythmogenic right ventricular cardiomyopathy (ARVC), where acute inflammation and myocardial injury suggest an active phase of the cardiomyopathy [17–19]. Additionally, myocardial inflammation, indicative of myocarditis, has also been detected in cardiomyopathies [20–22], as heart failure itself can provoke inflammation [21–23], creating a complex overlap between these entities and blurring diagnostic distinctions [9,24].

This study aims to investigate the prevalence of P/LP variants in pediatric patients with infarct-like myocarditis and explore whether clinical or imaging findings at diagnosis or during follow-up can predict the presence of a genetic background.

2. Methods

This prospective, multicenter study was conducted across five hospitals in Catalunya, Spain, over eight years (January 2016 to December 2024). Consecutive pediatric patients admitted with infarct-like myocarditis were included. Myocarditis was diagnosed according to the 2021 American Heart Association (AHA) Scientific Statement for children [25]. All patients were clinically suspected CMR-confirmed, using the 2018 Lake Louise criteria [6]. Endomyocardial biopsy was not performed, in line with current pediatric practice for stable cases with typical imaging findings. Patients with congenital structural heart defects, syndromic disorders, or metabolic, mitochondrial, or neuromuscular diseases were excluded, as well as those with complicated forms of myocarditis.

Clinical and epidemiological data were collected, with particular focus on family history of heart transplantation, sudden cardiac death, or unexplained cardiomyopathy. Fig. 1 illustrates the study flowchart for patient inclusion and follow-up.

Standard 12-lead electrocardiograms (ECG) were performed at admission and evaluated by a pediatric cardiologist. Transthoracic echocardiography was systematically conducted at diagnosis by experienced pediatric cardiologists, assessing LV systolic function, dimensions, and wall thickness. LV dysfunction was defined as a Simpson-derived LV ejection fraction (LVEF) <55 %. Ventricular dilatation and hypertrophy were defined as a *Z*-score > +2 for age and body surface area. Left atrial enlargement was defined as an indexed LA volume > 34 mL/m^2. Right ventricular (RV) dysfunction was defined as a fractional area change (FAC) < 35 % and tricuspid annular plane systolic excursion (TAPSE) z-score < −2, adjusted for age.

CMR was performed at diagnosis and after 12 months of follow-up, using a 1.5-T system (Avanto, software releases VB17 and VE11B; Siemens Medical Solutions) with cardiac synchronization. Steady-state-free precession (SSFP) sequences in a short-axis stack were utilized to assess heart function and volumes. T2-weighted short-tau inversion recovery (T2W-STIR) and T1-weighted turbo spin-echo (TSE) sequences were obtained before and after intravenous administration of gadolinium. Phase-sensitive inversion recovery (PSIR) reconstructions were performed 10 min after the injection of 0.15 mmol/kg gadolinium to evaluate late gadolinium enhancement (LGE). Tissue mappings included native T1 relaxation times (nT1), extracellular volume (ECV), and T2 relaxation times, measured on the LV posterior wall and the interventricular septum during end-diastole in short-axis views. T1-mapping was performed using a modified look-locker inversion recovery (MOLLI) sequence both pre-contrast and 15 min post-contrast. T2 maps were obtained during an 11-s end-expiratory breath-hold. Regional abnormalities were assessed using the AHA standardized 17-segment LV model [26]. The presence of a ring-like pattern was defined as an extensive subepicardial LGE distribution involving ≥3 contiguous segments in the same short-axis slice.

Genetic testing was carried out using the TruSight Cardio Panel (Illumina, San Diego, Ca, USA) using Next Generation Sequencing (NGS) technology and covering exonic and flanking intronic regions of 174 genes associated with cardiovascular diseases (see Supplementary Table S1 for complete variant list). Variants identified through NGS were validated using Sanger sequencing. Pathogenicity was assessed following the guidelines of the American College of Medical Genetics and Genomics (ACMG) [27] and the recommendations issued by the Clinical Genome Resource (ClinGen) Sequence Variant Interpretation Working Group [28].

Statistical analysis was performed using SPSS 25.0 (IBM). Proportions were used to describe nominal data, while medians and interquartile ranges (IQR) were used for continuous variables, as the sample did not follow a normal distribution. The Mann-Whitney *U* test was used for continuous variable comparisons. A *p*-value <0.05 was considered

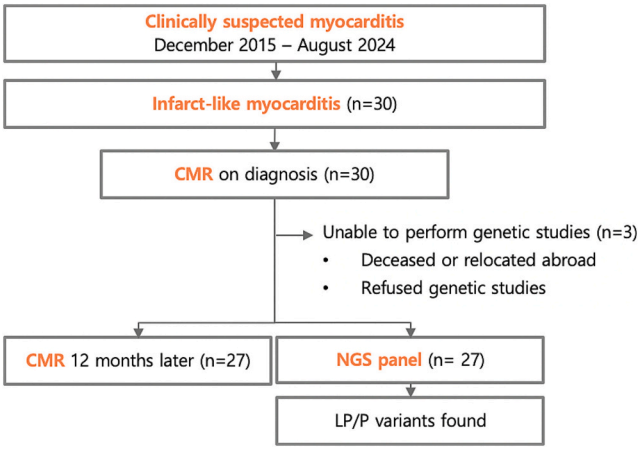

Fig. 1. Flowchart of the study. *Abbreviations*: Cardiac Magnetic Resonance (CMR), Next Generation Sequencing (NGS), Pathogenic/ Likely pathogenic (P/LP).

statistically significant. The corresponding author had full access to all the data in the study and takes responsibility for its integrity and the accuracy of the data analysis.

Ethical approval for this study was granted by the local ethics committee, ensuring compliance with ethical standards and the protection of participants' rights and safety. All activities conducted in this research adhered to the ethical principles outlined in the 1975 Declaration of Helsinki. Informed consent for publication was obtained from the parents of each patient.

3. Results

During an 8-year period, 30 adolescents (24 males; 80 %) with infarct-like myocarditis were included, with a median age of 14 years (IQR 12–16). None of the patients had prior personal or family history of cardiomyopathy, sudden cardiac death, or ventricular arrhythmia, although two patients reported a parental history of myocarditis.

Clinical and epidemiological data are presented in Table 1. Notably, 10 out of 30 patients (33 %) were admitted to the intensive care unit (ICU) for monitoring purposes despite having an uncomplicated clinical course, reflecting center-specific protocols rather than disease severity. All ICU-admitted patients were monitored with continuous telemetry, allowing for arrhythmic surveillance. Only two patients exhibited rhythm disturbances: one had isolated premature ventricular contractions likely originating from the RV outflow tract, and another experienced a brief, asymptomatic six-beat run of non-sustained ventricular tachycardia, with no recurrence. No additional arrhythmias were identified during follow-up.

A potential infectious trigger, typically an upper respiratory tract infection or gastroenteritis, was identified in 24/30 patients (80 %), with a median of 3 days prior to symptom onset (IQR 2–5). A causative microorganism was confirmed in 10 cases (33.3 %), as detailed in Supplementary Table S2, which includes the identified pathogens, diagnostic methods, and clinical context. Although the study period overlapped with the COVID-19 pandemic, all patients were systematically tested for SARS-CoV-2 on admission. Only two patients tested positive, and no cases were temporally related to COVID-19 vaccination.

Baseline CMR was performed a median of 3 days after clinical diagnosis (IQR 1–6.25), with all patients fulfilling the 2018 Lake Louise Criteria. Median LVEF was 60 % (IQR 50.25–62.75), with mild dysfunction observed in 8 patients (26.7 %). The median extent of LGE, expressed as the number of affected segments, was 5 (IQR 2–5). Parametric mappings were performed in 23 patients (76.7 %) due to its availability starting in October 2017. Detailed imaging findings at diagnosis are presented in Table 1, and the segmental distribution of LGE is illustrated in Fig. 2.

Of the 27 patients (90 %) who underwent NGS testing, 7 (25.9 %) had P/LP variants, one patient having 2 P/LP variants. Six patients (22.2 %) presented variants in cardiomyopathy-related genes and one patient had an incidental *KCNH2* variant and subsequently displayed electrocardiographic features consistent with long QT syndrome. The remaining patients had either non-conclusive results or variants of uncertain significance. Identified variants, their molecular consequences, and ACMG classification are presented in Table 2 and illustrated in Fig. 3. Segregation studies enabled identification of the parental origin of each variant. Although all carrier parents were asymptomatic at the time of evaluation, family screening revealed LV hypertrophy in the *MYBPC3* carrier and prolonged QTC interval in the *KCNH2* carrier. CMR studies are planned to further assess for potential subclinical disease in the remaining genotype-positive parents.

The median follow-up time was 3 years (IQR 2–7). During follow-up, 9 patients (30 %) experienced a recurrence of myocarditis, none of which were associated with heart failure or arrhythmic complications. The median time to the first recurrence was 2.5 years (IQR 1.08–4 years). One patient, later found to carry a variant in *ALPK3,* reported 3 episodes of chest pain. Although a potential association with the

Table 1
Clinical, demographic, laboratory, electrocardiographic, and imaging findings of adolescents with infarct-like myocarditis. Quantitative data are presented as n (%), and qualitative data as median (interquartile range). Local normal values for CMR tissue mappings are <1059 ms for native T1, < 30 % for extracellular volume and < 50 ms for T2 mapping. *Abbreviations*: Family history (FH); Intensive Care Unit (ICU); High-sensitivity troponin T (hs-TnT); Creatine kinase (CK); N-terminal pro b-type Natriuretic Peptide (NT-proBNP), C-reactive protein (CRP), Non-sustained ventricular tachycardia (NSVT), Left ventricle (LV), LV ejection fraction (LVEF); Left atrium (LA); Right ventricle (RV); Cardiac Magnetic Resonance (CMR); End-diastolic volume (EDV); Late gadolinium enhancement (LGE).

	TOTAL ($n = 30$)	P/LP variants) $n = 6$	No P/LP variants $n = 21$	Statistical significance (p-value)
Demographics				
	14 (4)	14 (2)	15 (4)	0.842
	24 (80 %)	4 (66.7 %)	18 (85.7 %)	0.303
- Age, years	2 (6.7 %)	0 (0 %)	2 (9.5 %)	1
- Males	9 (30 %)	4 (66.7 %)	5 (23.8 %)	***0.073***
- FH of myocarditis	10 (33.3 %)	1 (16.7 %)	7 (33.3 %)	0.633
- Prior episode of myocarditis				
- ICU admission				
Clinical presentation				
	24 (80 %)	5 (83.3 %)	16 (76.2 %)	0.596
	30 (100 %)	6 (100 %)	21 (100 %)	-
- Recent viral history	0 (0 %)	0 (0 %)	0 (0 %)	-
- Chest pain	2 (6.7 %)	2 (33.3 %)	2 (9.5 %)	1
- Syncope	9 (30 %)		7 (33.3 %)	1
- Palpitations				
- Dyspnea				
Laboratory findings				
	9205 (3332.5)	9205 (6755)	9410 (3095)	0.932
	1667.5 (1737.5)	1446 (2630.75)	1671 (1600.5)	0.820
- Leucocytes/mm2	434 (580)	980.5 (1508)	426.5 (462.75)	0.139
- Hs-TnT, ng/L	779 (771.75)	269 (745.9)	839.5 (965.25)	0.174
- CK, UI/L	3.53 (4.55)	0.83 (7.3)	3.8 (3.75)	0.195
- NT-proBNP, ng/L	10 (33.3 %)	1 (16.7 %)	8 (38.1 %)	0.628
- CRP, mg/dL				
- Microorganism identification				
Electrocardiogram				
	29 (96.7 %)	6 (100 %)	20 (95.2 %)	0.778
	7 (23.3 %)	0 (0 %)	7 (33.3 %)	0.155
- ST segment elevation	1 (3.3 %)	0 (0 %)	1 (4.8 %)	1
- Negative/flat T waves (lateral/ inferior)	0 (0 %)	1 (16.7 %)	0 (0 %)	-
- Q waves	4 (13.3 %)	0 (0 %)	3 (14.3 %)	0.659
- Wide QRS complex	0 (0 %)	0 (0 %)	0 (0 %)	-
- Low voltages	0 (0 %)		0 (0 %)	-
- Epsilon wave	2 (6.7 %)		2 (9.5 %)	1
- Atrioventricular blocks				
- Ventricular ectopy / NSVT				
Echocardiogram on admission				
	63.5 (11.75)	65.5 (10.25)	63 (15)	0.289
	7 (23.3 %)	0 (0 %)	6 (28.6 %)	0.284
- LVEF, %	2 (6.7 %)	0 (0 %)	2 (9.5 %)	1
- LV dysfunction (LVEF<50 %)	13 (43.3	3 (50 %) 1 (16.7 %)	10 (47.6 %)	0.638 0.402

(*continued on next page*)

Table 1 (*continued*)

	TOTAL (n = 30)	P/LP variants) n = 6	No P/LP variants n = 21	Statistical significance (p-value)
- LV dilation	%)	1 (16.7 %)	1 (4.8 %)	0.402
- LV hypertrophy	2 (6.7 %)	0 (0 %)	1 (4.8 %)	-
- LA dilation	3 (10 %)		0 (0 %)	
- Mitral regurgitation	0 (0 %)			
- RV dysfunction				
CMR parameters				
	3 (6.25)	2.5 (6.25)	3 (7.5)	0.629
	60 (12.5)	59 (11.25)	60 (19.5)	0.712
- Time to CMR, days	7 (23.3	1 (16.7 %)	6 (28.6	1
- LVEF, %	%)	2 (33.3 %)	%)	0.648
- LV dysfunction	14 (46.7	82 (28.5)	11 (52.4	0.387
(LVEF <50 %)	%)	58.5	%)	0.721
- Wall motion	85.5	(11.75)	87.5	0.378
abnormalities	(15.25)	90.5	(14.25)	0.455
- LV-EDV indexed,	58 (12)	(19.75)	57 (11.5)	-
ml/m2	83 (19)	(100 %)	81.5 (16)	***0.056***
- RVEF, %	27 (90 %)	6 (100 %)	18 (85.7	***0.025***
- RV-EDV indexed,	30 (100	3 (50 %)	%)	0.376
ml/m2	%)	3 (50 %)	21 (100	0.472
- Edema on T2-STIR	5 (16.7	5 (3.75)	%)	
sequences	%)	3 (50 %)	2 (9.5 %)	0.505
- Presence of LGE	4 (13.3		1 (4.8 %)	0.221
- Septal LGE	%)	1176	5 (3)	0.878
- Ring-like pattern	5 (3)	(352.25)	8 (38.1	
- Number of segments	11 (36.7	37.5 (28)	%)	
with LGE	%)	54 (17.25)		
- Pericardial effusion			1100.5	
- Tissue mappings	1097		(140)	
- Native T1 mapping,	(136)		27 (8)	
ms	27.5		57.5 (13)	
- Extracellular volume	(13.5)			
(ECV), %	57 (14)			
- T2 mapping, ms				

presence of P/LP variants in cardiomyopathy genes did not reach statistical significance (p = 0.073) in this small sample, recurrences were more frequent among those carrying P/LP variants (66.7 % vs. 23.8 %), which may be clinically relevant. One patient died from an unrelated oncological diagnosis, and one was lost to follow-up due to relocation abroad.

At the time of diagnosis, no significant associations were found between genetic findings and clinical symptoms, laboratory results, electrocardiographic abnormalities, or echocardiographic parameters. CMR at baseline showed no significant differences in ventricular dysfunction, ventricular volumes, or parametric mapping values between genetic-positive and genetic-negative patients (p-values are shown in Table 1). The extent of LGE (number of affected segments) was also not associated with genetic findings (p = 0.376), and its distribution was similar (Fig. 2). However, the presence of a ring-like LGE pattern on baseline CMR was significantly associated with positive genetic testing (p = 0.025), while septal involvement showed a p-value of 0.056 which did not meet the conventional threshold for significance (p < 0.05) in this small sample, though may be clinically relevant.

Follow-up CMR at 12 months revealed preserved LV function (median LVEF 60 %, IQR 55–63.5), normal LV volumes (median LVEDV 87.5 ml/m^2, IQR 76–93.5), with no significant differences between groups (p = 0.494). A reduction in the extent of radiological abnormalities was observed, with a median of 2 LGE-affected segments (IQR 1–4). The persistence of increased signal intensity in sequences sensitive to myocardial edema (T2-STIR) was significantly associated with positive genetic findings (p = 0.009). Furthermore, all genetic-positive patients demonstrated LGE persistence compared to 83.3 % of genetic-negative patients, though this difference did not reach statistical significance (p = 0.202). Notably, while the extent of LGE (segments affected) at baseline did not distinguish between genetic-positive and negative patients, it became statistically significant at 12 months (p = 0.047, Fig. 4), with a distinct distribution pattern illustrated in Fig. 5. No

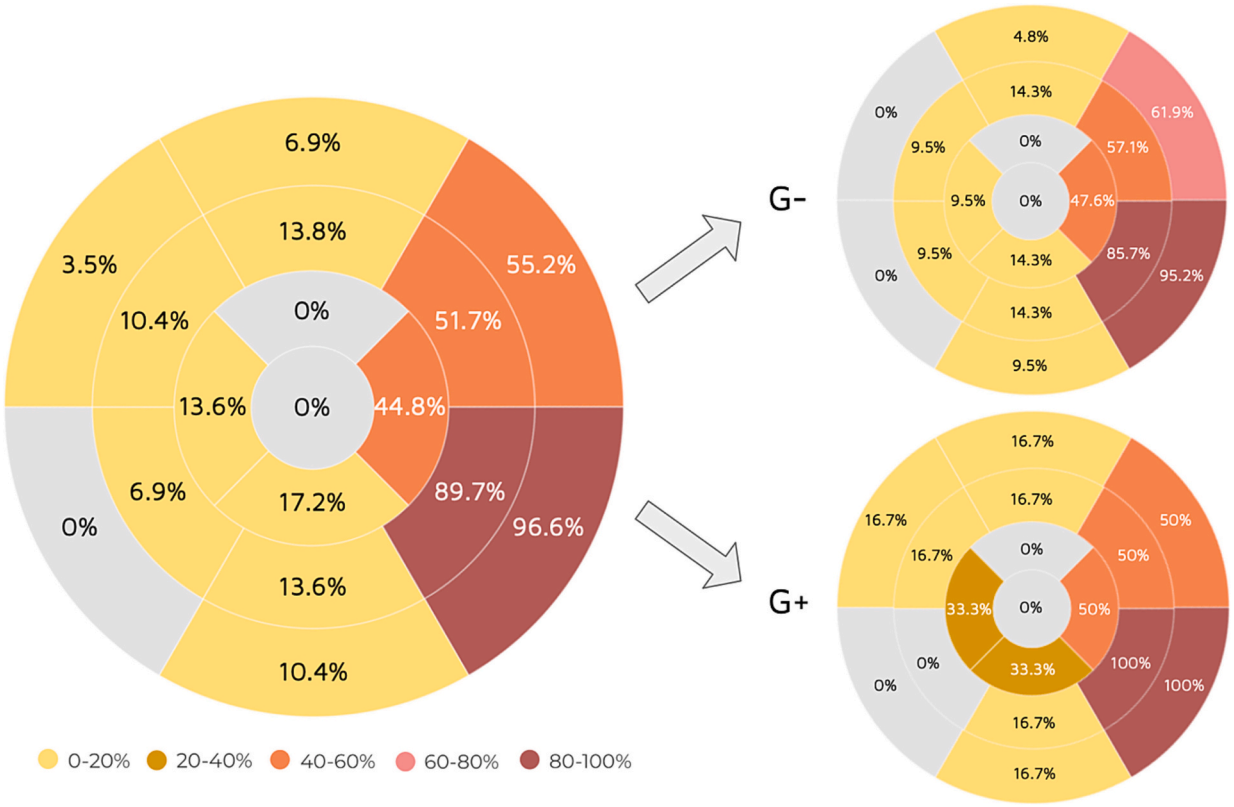

Fig. 2. Distribution of LGE at diagnosis according to the AHA standardized 17-segment LV model. The first circle illustrates the LGE distribution in the entire cohort, while the subsequent circles show the distribution in patients with positive genetic findings (G+) and those with negative findings (G-).

Table 2
Genetic variants detected in the cohort, detailing their molecular consequences, American College of Medical Genetics and Genomics (ACMG) classification, and the supporting evidence used for classification. Variants identified in patients 1–6 involve cardiomyopathy-related genes, whereas the variant in patient 7 was an incidental finding.

Patient	GEN	Genomic variant localization (GRCh37/hg19)	Transcript variant	Protein variant	Molecular consequence	ACMG/AMP classification	ACMG/AMP evidence	Origin
#1	*DSP*	chr6–7,565,682	NM_004415.4: c.868G > A	NP_004406.2: p. Glu290Lys	Missense	Likely pathogenic	PS4; PM2_ supporting; PP3_ moderate	Maternal
#2	*MYBPC3*	chr11–47,372,137	NM_000256.3: c.309_321dup	NP_000247.2: p. Pro108Alafs*9	Frameshift	Pathogenic	PVS1; PS2; PM2_Supporting; PP3	Paternal
#3	*ALPK3*	chr15–85,400,404	NM_020778.5: c.2437dup	NP_065829.4: p. Val813GlyfsTer66	Frameshift	Likely pathogenic	PVS1; PM2_Supporting	Paternal
#4	*DSG2*	ch18–29,101,061	NM_001943.5: c.379-1G > A	–	Splice acceptor	Pathogenic	PVS1; PM2_Supporting	Maternal
	TTN	chr2–179,648,796	NM_001267550.2: c.2775 + 1G > T	–	Splice donor	Likely pathogenic	PVS1_Moderate; PS4_Moderate; PM2_Supporting	Paternal
#5	*PKP2*	chr12–33,031,915	NM_001005242.3: c.275 T > A	NP_001005242.2:p. Leu92Ter	Nonsense	Pathogenic	PVS1; PS4; PM2_Moderate	Paternal
#6	*DSP*	ch6–7,580,207	NM_004415.4: c.3784C > T	NP:004406.2: p. Gln1262Ter	Nonsense	Likely Pathogenic	PVS1; PM2_Supporting	Maternal
#7	*KCNH2*	chr7–150,648,737	NM_000238.4: c.1744C > T	NP_000229.1: p. Arg582Cys	Missense	Pathogenic	PM6_Very strong; PM1; PM2; PM5; PP2; PP3	Maternal

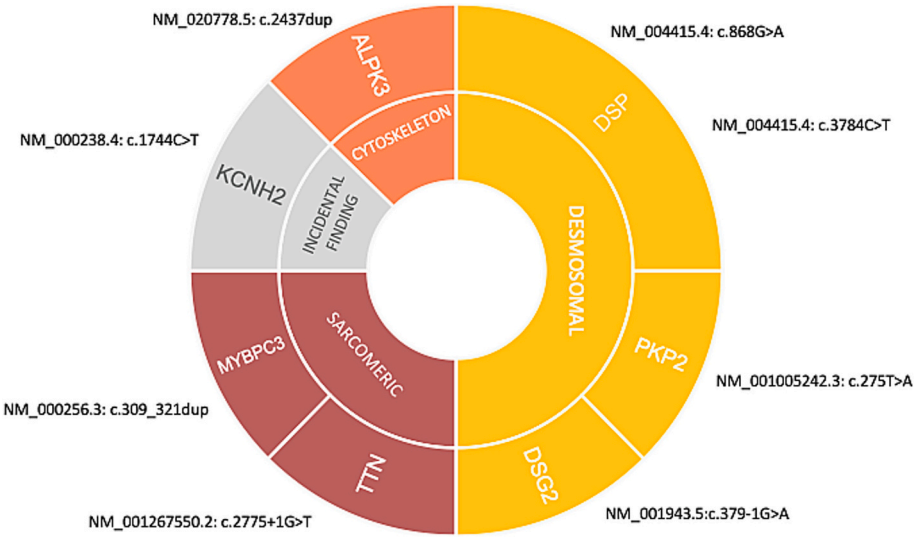

Fig. 3. Classification of the detected variants.

significant associations were found with tissue mapping parameters (*p*-values for nT1: 0.775; ECV: 0.383; T2 mappings: 0.703).

4. Discussion

In this prospective multicenter study, we investigated the genetic and imaging characteristics of adolescents with infarct-like myocarditis, identifying key associations at diagnosis and during follow-up. Genetic testing revealed P/LP variants in cardiomyopathy-related genes in 22.2 % of patients. Regarding baseline CMR findings, while neither the extent of LGE nor ventricular dysfunction were associated with positive genetic testing, the distribution of LGE (ring-like LGE pattern and septal involvement) was related to genetic positivity. During follow-up, myocarditis recurred in 30 % of patients, with a higher frequency among those with P/LP variants. At 12-month follow-up, CMR demonstrated a reduction in the extent of LGE across the cohort, with a significant difference between genetic-positive and genetic-negative patients, suggesting its potential utility as a marker of genetic predisposition.

The prevalence of P/LP variants in our cohort is higher than previously reported [10–13]. Understanding the genetic background of myocarditis remains challenging due to the extreme heterogeneity of presentations and diagnostic methods across published studies [29]. Most prior research has primarily focused on adults with complicated presentations, often identifying variants in sarcomeric or desmosomal genes [8–10]. In contrast, the high prevalence of P/LP variants in our relatively homogeneous group of adolescents with uncomplicated myocarditis underscores the importance of genetic testing in pediatric populations, even in cases without severe clinical presentations.

Some factors may account for the higher diagnostic yield in our study. Genetic susceptibility could play a more prominent role in pediatric myocarditis than in adults, particularly as an early manifestation of inherited cardiomyopathies [18]. Additionally, the use of a broad NGS panel may have increased variant detection compared to prior studies using narrower panels [12,13], although all identified variants were located in genes commonly included in most cardiomyopathy-focused panels. Lastly, restricting the cohort to infarct-like presentations ensured a high degree of phenotypic homogeneity, facilitating the identification of genotype–phenotype associations.

The potential overlap between myocarditis and inherited

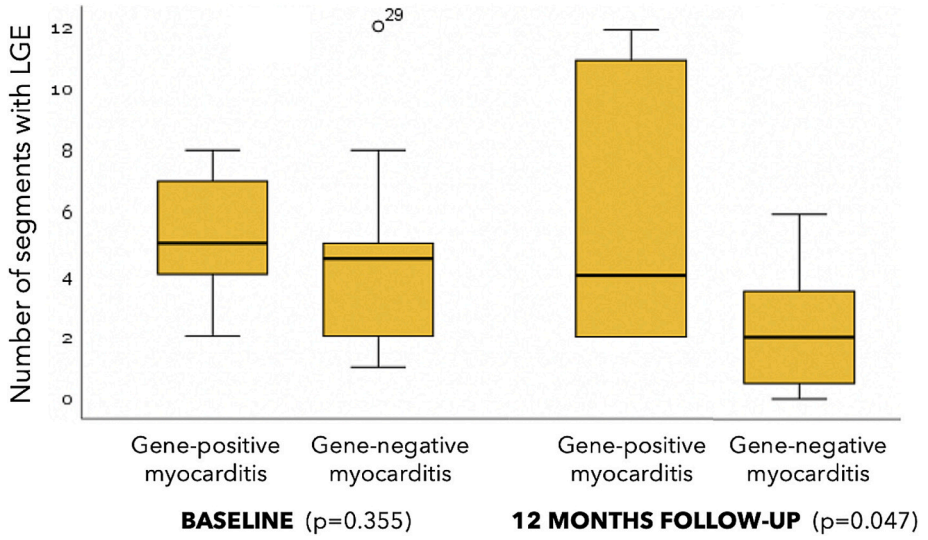

Fig. 4. Comparison of LGE extent (number of affected segments) at baseline and 12-month follow-up in patients with positive and negative genetic findings. Boxplots illustrate the reduction in LGE extent over time in gene-negative patients and the increase in those with positive results.

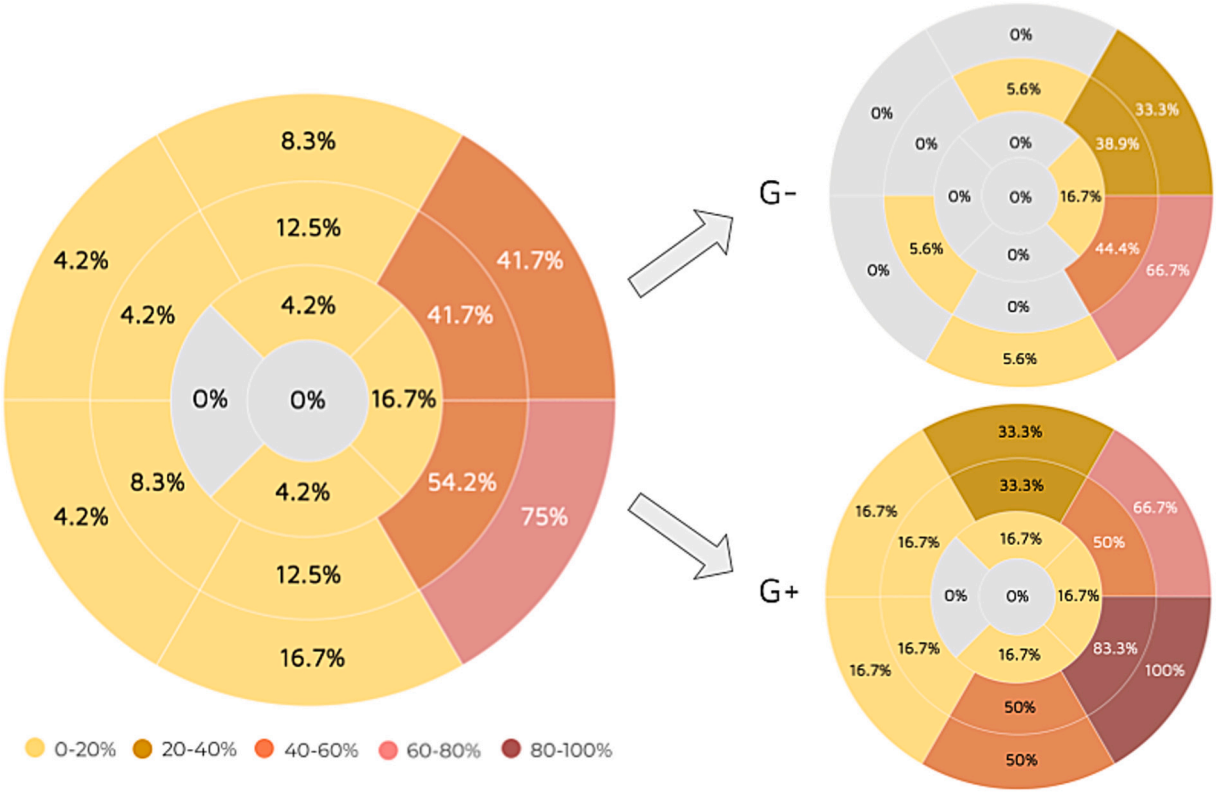

Fig. 5. Distribution of LGE at 12-months follow-up according to the AHA standardized 17-segment LV model. The first circle illustrates the LGE distribution in the entire cohort, while the subsequent circles show the distribution in patients with positive genetic findings (G+) and those with negative findings (G-).

cardiomyopathies represents a diagnostic challenge, considering that chest pain with ECG abnormalities, troponin release and LGE are present in both conditions [18,30]. This is especially relevant in adolescence, when the initial manifestations of cardiomyopathies often arise [3]. This overlap is well-documented in conditions such as NDLVC, ARVC, and DCM, where inflammatory episodes may represent "hot phases" of the underlying cardiomyopathy [18,19,30]. While this association is rarely reported with hypertrophic cardiomyopathy, we describe a patient initially diagnosed with myocarditis who carried a pathogenic variant in *MYBPC3* and developed this cardiomyopathy two years later. A notable example in our cohort was a patient with myocarditis who had an incidental finding of a pathogenic *KCNH2* variant and later developed ECG features of long QT syndrome; both diagnoses were identified and are under surveillance. Identification of prolonged QT intervals is clinically significant, not only as a marker of arrhythmogenic risk but also because it is associated with poorer outcomes in myocarditis [20].

A recent viral history or detection of a microbiological agent was not associated to the absence of genetic findings in our study. While uncomplicated myocarditis in a viral context may seem entirely acquired, viral infections have been described as a potential second hit in

genetically predisposed individuals, acting as a trigger for myocardial affection [9,15,16]. The interaction between genetic predisposition and viral infections remains under investigation and could guide decisions on immunomodulatory therapies in myocarditis [29,31].

Our findings suggest that genetic predisposition may manifest through recurrent inflammatory episodes, although statistical significance was not achieved ($p = 0.073$), likely due to the limited sample size. Recurrent myocarditis, previously associated in adult studies with mutations in ARVC and NDLVC-related genes, such as *DSP* and *PKP2,* may represent early inflammatory episodes of an underlying cardiomyopathy that evolves over time [17,19,32]. These results highlight the importance of longitudinal monitoring and early preventive measures [17,18], alongside the utility of genetic testing in these patients [4].

All diagnoses in our study were confirmed by CMR, adhering to the 2018 Lake Louise Criteria [6], ensuring a standardized and reliable diagnostic approach. While no significant differences were observed in ventricular function, volumes, or parametric mapping values between genetic-positive and genetic-negative patients, the presence of a ring-like LGE pattern and septal involvement were identified as factors associated with positive genetic findings. These patterns have been linked to adverse outcomes in myocarditis and to inherited cardiomyopathies [7,8,22]. Evaluating the extent and distribution of LGE at diagnosis offers important diagnostic and prognostic value.

Follow-up CMR at 12 months demonstrated significant improvements across the cohort. However, genetic-positive patients exhibited a greater LGE burden ($p = 0.047$), suggesting a potential influence of genetic predisposition on myocardial remodeling over time [8]. Additionally, T2-STIR sequences indicative of myocardial edema remained abnormal in some genetic-positive patients ($p = 0.009$), highlighting ongoing inflammation [33]. These findings align with prior studies indicating that persistent scarring and inflammation may reflect early phases of chronic cardiomyopathies, particularly NDLVC [8,22,33]. Longitudinal CMR serves not only to monitor myocardial recovery but also to identify patients at increased risk for long-term complications, facilitating risk stratification [6,33].

Risk stratification for future adverse cardiovascular events after myocarditis is particularly challenging in younger patients, given the longer life span over which potential complications may arise [34]. While many patients experience spontaneous recovery, persistent inflammation or scarring may predispose them to adverse outcomes, including arrhythmias and ventricular dysfunction [31,24,22]. Our study emphasizes the importance of continued follow-up and proactive management, even in uncomplicated cases with preserved ventricular function. Furthermore, recognizing an early phase of cardiomyopathy has significant implications for management, including sudden death risk stratification, exercise recommendations, and cascade screening of family members to identify at-risk relatives [8,19]. Long-term studies are needed to further evaluate cardiovascular outcomes in uncomplicated myocarditis.

4.1. Limitations

This study has some limitations to consider. It was conducted in a limited number of centers, which may introduce selection bias and limit the generalizability of the findings to other populations. The relatively small cohort size, a challenge inherent to pediatric studies, could have influenced the prevalence estimates of P/LP variants. This issue has been highlighted in previous studies, where smaller cohorts with high variant rates were supposed to affect prevalence estimates in meta-analyses [29]. Additionally, routine endomyocardial biopsy was not performed, following current guidelines that reserve biopsies for high-risk cases. This may have introduced a potential misclassification bias, as some cases classified as myocarditis might actually represent cardiomyopathies. Despite these limitations, this study provides valuable insights into the genetic characteristics of infarct-like myocarditis in adolescents. Larger registries and multicenter collaborations will help further validate and expand upon these findings.

5. Conclusions

Our study highlights the significant presence of P/LP variants in cardiomyopathy-related genes among adolescents with infarct-like myocarditis. Specific CMR findings, such as a ring-like LGE pattern and septal involvement, were associated with genetic predisposition, underscoring the critical role of advanced imaging in the diagnostic and prognostic evaluation of myocarditis. Furthermore, the persistence of edema on T2-STIR sequences and the more extensive LGE observed at follow-up suggest ongoing myocardial remodeling in genetic-positive patients. Recurrences of myocarditis were more frequent among patients with P/LP variants, suggesting that recurrent myocarditis may arise from genetically susceptible myocardium or represent inflammatory episodes of an evolving cardiomyopathy. These findings support the need for continued follow-up even in cases of uncomplicated myocarditis, guiding individualized management strategies, including risk stratification, exercise recommendations, and family screening, to improve long-term outcomes.

This statement is to certify that all authors have seen and approved the manuscript.

being submitted, have contributed significantly to the work, attest to the validity and legitimacy of the data and its interpretation, and agree to its submission to the *International Journal of Cardiology*.

We attest that the article is the Authors' original work, has not received prior publication and is not under consideration for publication elsewhere. We adhere to the statement of ethical publishing as appears in the International of Cardiology (citable as: Shewan LG, Rosano GMC, Henein MY, Coats AJS. A statement on ethical standards in publishing scientific articles in the International Journal of Cardiology family of journals. Int. J. Cardiol. 170 (2014) 253–254 DOI:https://doi.org/10.1016/j.ijcard.2013.11).

On behalf of all Co-Authors, the corresponding Author shall bear full responsibility for the submission. Any changes to the list of authors, including changes in order, additions or removals will require the submission of a new author agreement form approved and signed by all the original and added submitting authors.

All authors are requested to disclose any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work. If there are no conflicts of interest, the COI should read: "The authors report no relationships that could be construed as a conflict of interest".

CRediT authorship contribution statement

Roger Esmel-Vilomara: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lucía Riaza:** Methodology, Investigation, Data curation. **Paola Dolader:** Validation, Methodology, Investigation. **Benjamín Rodríguez-Santiago:** Writing – review & editing, Investigation, Data curation. **Amaia Lasa-Aranzasti:** Validation, Formal analysis, Conceptualization. **Patricia Muñoz-Cabello:** Methodology, Formal analysis. **Paula Fernández-Álvarez:** Supervision, Investigation. **Marc Figueras-Coll:** Data curation. **Lisa Bianco:** Data curation. **Andrea Bueno-Gómez:** Investigation. **Laura Vargas-Pons:** Data curation. **Elisabet Camprubí-Tubella:** Data curation. **Cristina Marimon-Blanch:** Data curation. **Anna Sabaté-Rotés:** Writing – review & editing, Validation, Supervision. **Ferran Rosés-Noguer:** Writing – review & editing, Validation, Supervision. **Ferran Gran:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization.

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Declaration of competing interest

Authors have no relevant conflicts of interests to disclose.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2025.133255.

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Esmel-Vilomara R, Figueras-Coll M, Rosés-Noguer F, Balcells J, Gran F.

Journal:

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NINTH ARTICLE

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Advancements and challenges in pediatric dilated cardiomyopathy: a comprehensive review of current approaches and future directions.

Authors:

López-Guillén JL, Esmel-Vilomara R (co-first author), Rodoman I, Duarte-Constante A, Kendall S, Freixa-Benavente A, Díaz D, Cantarutti N, Moscatelli S, Jeewa A.

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DISCUSSION

DISCUSSION

This thesis explored the aetiological spectrum, diagnostic challenges, and prognostic factors of acute myocarditis in the paediatric population, focusing on the potential role of early diagnostic tools in improving clinical management and outcomes. Given the complexity and heterogeneity of myocarditis presentations in children, the discussion has been structured around the main hypotheses proposed at the beginning of this work, highlighting the novel contributions of this thesis, and identifying remaining gaps and future research directions.

<u>HYPOTHESIS 1:</u> A substantial proportion of paediatric patients diagnosed with uncomplicated myocarditis carry P/LP variants in cardiomyopathy-associated genes, even in the absence of family history or prior cardiac disease.

In recent years, growing evidence has suggested that genetic predisposition plays a significant role in the development and clinical course of myocarditis, challenging the traditional perception of this condition as purely acquired and triggered by infectious, immune-mediated, or toxic insults [47–51]. This thesis investigated the genetic background of myocarditis. We identified through a prospective multicentre study, P/LP variants in cardiomyopathy-associated genes in 22.2% of adolescents with infarct-like myocarditis, despite the absence of prior family history or known cardiac disease. Restricting the cohort to infarct-like myocarditis ensured a higher degree of clinical homogeneity, potentially facilitating clearer genotype-phenotype associations.

Previous research has reported substantial variability in the prevalence of genetic variants among patients with myocarditis, ranging from 4% in uncomplicated forms to over 20% in cases presenting with heart failure or arrhythmias [47,53,56,57]. Higher proportions, reaching up to 45%, have been documented in subgroup analyses focusing on children with severe clinical manifestations, such as acute heart failure or life-threatening arrhythmias, while remaining lower (4.2%) in patients with less complicated presentations [47]. However, most of these estimates are derived from adult cohorts or from patients with complicated disease, and data specifically addressing the paediatric population remain scarce, particularly among patients presenting with infarct-like forms that are often considered clinically benign [53,57,58]. Our study contributes to addressing this gap, suggesting that the contribution of genetic factors may be

underestimated in adolescents with myocarditis, especially when the presentation is not severe. The high prevalence of genetic variants identified within our homogeneous adolescent cohort underscores the importance of considering genetic testing more broadly in this context, advocating for its integration not only in fulminant cases but also in those presenting with apparently uncomplicated forms.

The high diagnostic yield observed in our study may also be partially explained by the use of a broad NGS panel, improving variant detection compared to earlier studies with narrower panels [53,57]. Nonetheless, all identified variants were located in well-recognized cardiomyopathy genes included in most standard testing strategies.

In this context, episodes of myocarditis may represent an early inflammatory stage, or "hot phase," in the natural history of inherited cardiomyopathies, particularly DCM, ARVC, or NDLVC [50,63,66]. Supporting this hypothesis, myocardial inflammation has been documented not only in classic myocarditis but also in genetically mediated cardiomyopathies [63,66], as well as in patients with heart failure, where secondary immune activation and inflammatory infiltrates may contribute to myocardial injury [62,68,70].

The clinical overlap between myocarditis and inherited cardiomyopathies represents a significant diagnostic challenge. Shared features, such as chest pain, ECG abnormalities, troponin elevation, and LGE on CMR, complicate the differentiation between these two entities [63,67]. This overlap is particularly concerning during adolescence, a critical period when initial manifestations of many inherited cardiomyopathies emerge [1].

Recurrent myocarditis episodes may serve as an additional clinical marker for underlying genetic predisposition. In our cohort, although statistical significance was not reached ($p = 0.073$), recurrences were more frequent in those harbouring P/LP variants. This observation is consistent with previous adult studies linking recurrent myocarditis to desmosomal gene variants, particularly in *DSP* and *PKP2*, supporting the theory that these episodes could represent the inflammatory manifestation of an evolving cardiomyopathy [50,65,66]. Truncating variants in *DSP* are the most frequently reported variants in patients with preserved systolic function and infarct-like presentation [49–51]. Supporting this, previous studies have shown that approximately 15% of patients with known *DSP* variants experience myocarditis-like episodes, reinforcing the concept of myocarditis as a potential early expression of desmosomal cardiomyopathy [60].

In conclusion, our research strengthens the growing body of evidence suggesting that genetic predisposition is not restricted to complicated myocarditis but may also play a critical role in paediatric patients with uncomplicated myocarditis. These findings support the systematic incorporation of genetic testing in the diagnostic evaluation of all paediatric patients with myocarditis, independently of initial severity, to facilitate early risk stratification, guide family screening, and enable timely therapeutic decisions. Moreover, they highlight the importance of maintaining long-term cardiological follow-up, to detect late-onset complications.

HYPOTHESIS 2: Genetic testing plays a pivotal role in the diagnostic evaluation of paediatric patients with new-onset heart failure.

New-onset heart failure in infants may result from viral infections, an underlying genetic cardiomyopathy, or even a combination of both mechanisms [4,51]. Distinguishing between complicated myocarditis and primary genetic cardiomyopathies in infants presenting with new-onset heart failure remains a significant diagnostic challenge [57,69].

In this study, we describe a series of infants presenting with early-onset cardiac dysfunction, a severe clinical scenario where accurate assessment plays a key role in guiding care. Currently, evidence comparing the utility of EMB, CMR, and genetic testing in this specific age group remains limited. In this context, our study highlights the limitations of conventional diagnostic tools, as both CMR and EMB consistently demonstrated myocardial inflammation in all patients, regardless of their underlying aetiology. Despite fulfilling the established diagnostic criteria for myocarditis on both CMR and EMB [4], the clinical evolution of these patients was markedly different depending on their genetic background.

The detection of P/LP variants in cardiomyopathy-associated genes was associated with worse outcomes, including persistent ventricular dysfunction and progression to heart transplantation. These findings support previous hypotheses suggesting that genetic predisposition may increase myocardial vulnerability to environmental triggers such as viral infections or immune-mediated injury [54,55], potentially acting as a “second hit” that exacerbates myocardial damage in susceptible individuals [48,52]. In individuals with an underlying genetic susceptibility, myocardial oedema, endothelial dysfunction, fibrosis, and immune system dysregulation may contribute to the development of ventricular dysfunction [51,67].

At the same time, inflammation detected by EMB is not exclusive to virus-mediated myocarditis, as inflammatory infiltrates may also appear as a secondary response to heart failure itself [62,70,110] and also in the context of genetically mediated cardiomyopathies [63,66] as an early sign of disease progression [68,72]

Given this overlap, relying solely on clinical presentation, imaging, or histology may lead to diagnostic uncertainty, particularly in infants where robust prospective data remain scarce. In our experience, genetic testing played an important role in clarifying the aetiology of these cases, especially when traditional investigations were inconclusive. Its integration into the diagnostic pathway not only allows for a more precise aetiological diagnosis but also has important clinical implications for family screening, arrhythmic risk stratification, and long-term management.

Although current guidelines do not universally recommend routine genetic testing in myocarditis [56], our findings, together with emerging evidence from other studies [55,57,63], support its consideration in infants with new-onset heart failure, particularly in severe or refractory cases, virus-negative and in those with family history or recurrent episodes. A comprehensive diagnostic strategy that incorporates genetic analysis alongside CMR, EMB, and microbiological testing may be the best approach to improve diagnostic accuracy and guide prognosis in this complex clinical scenario.

<u>HYPOTHESIS 3</u>: Standard diagnostic tools, such as the 12-lead electrocardiogram, may offer early indicators of specific viral aetiologies, particularly PVB19.

The 12-lead ECG remains an essential first-line tool in the evaluation of patients with suspected myocarditis [1,4,189]. While previous studies consistently report ECG abnormalities in nearly all cases of myocarditis [90,91], the potential diagnostic value of specific ECG patterns in relation to the underlying viral aetiology has not been thoroughly explored, particularly in paediatric populations where myocarditis is less frequent and cohort sizes are often limited.

In this context, our study identifies for the first time a distinctive ECG pattern associated with PVB19 myocarditis in children. The pattern, characterised by the presence of peaked P waves, low QRS voltages, and repolarization abnormalities (including flattened or inverted T waves, with or without QTc prolongation), demonstrated a high specificity (98%) and moderate

sensitivity (70%) for PVB19 detection, offering a potential non-invasive clue for early diagnostic orientation.

The high prevalence of repolarization abnormalities observed in our PVB19 cohort (100%), contrasts with the 35.1% seen in myocarditis of other viral origins, which is consistent with previously reported rates of T-wave abnormalities in myocarditis (9-48%, depending on the severity of presentation) [90,91,95]. These repolarization changes are believed to reflect underlying myocardial inflammation and oedema, creating inhomogeneity in repolarization across the myocardial wall, particularly when subepicardial involvement delays repolarization relative to the endocardium [90,95,190]. Repolarization abnormalities have been correlated in earlier studies with oedema and necrosis extension on CMR [95], supporting this mechanistic link.

Similarly, the finding of low QRS voltages in 80% of PVB19 patients in our cohort exceeds the prevalence reported in genetic cardiomyopathies, ranging from 3–6% in DCM to up to 41% in NDLVC [190]. Beyond known causes such as pericardial effusion, obesity or emphysema [91,190], expansion of extracellular space due to myocardial oedema and fibrosis likely contributes to this phenomenon [90]. Finally, 70% presented peaked P waves, potentially explained by elevated atrial pressures secondary to diastolic dysfunction and restrictive filling pattern induced by myocardial inflammation [190].

While the ECG alone lacks sufficient sensitivity and specificity for the definitive diagnosis of myocarditis [90,189], the identification of this distinctive pattern at presentation could raise early suspicion of PVB19 infection and guide subsequent diagnostic steps. This approach may be particularly valuable in settings where access to advanced diagnostic tools, such as CMR or EMB, is limited. Although current clinical guidelines do not support the initiation of pathogen-specific therapies based solely on ECG findings, early empirical immunomodulatory treatment with corticosteroids may be considered in complicated cases of myocarditis, especially in fulminant presentations [1,4]. The presence of this specific ECG pattern may help support such empirical decision-making in selected scenarios. Moreover, immunosuppressive regimens combined with interferon-beta therapy have been proposed as a strategy to suppress active PVB19 transcription and reduce myocardial injury in PVB19 myocarditis [24,28,156], therefore, the early identification of severely affected patients with an ECG suspicion of PVB19 involvement could help anticipate the need for targeted therapeutic interventions.

If validated in larger, multicentre cohorts, this ECG pattern could serve as a simple, accessible screening tool to aid in early viral aetiology identification, facilitating more rapid diagnostic orientation, optimized testing strategies, and potentially earlier intervention for paediatric patients with myocarditis.

<u>HYPOTHESIS 4:</u> Cardiac magnetic resonance imaging performed at the time of presentation may not reliably differentiate between virus-mediated myocarditis and genetically mediated cardiomyopathies in infants presenting with acute heart failure. Similarly, the presence of myocardial inflammation detected by endomyocardial biopsy and defined by the Marburg immunohistochemical criteria may not be specific for myocarditis.

The assessment of infants with acute heart failure presents a major diagnostic challenge, particularly when attempting to distinguish between virus-mediated myocarditis and genetically determined cardiomyopathies at the time of initial presentation. While both CMR and EMB remain the cornerstone investigations for evaluating myocardial inflammation, their precise role in defining the aetiology of heart failure in infants remains unclear, as very few studies have explored this question systematically in this age group [9,129,177].

CMR is widely recognized as the reference non-invasive modality for tissue characterization and quantification of myocardial inflammation in myocarditis, offering valuable information on myocardial oedema, fibrosis, and hyperaemia [3,122]. The diagnostic performance of CMR was notably improved following the introduction of the updated 2018 Lake Louise Criteria, increasing sensitivity and specificity to 87.5% and 96.2% [117,128], respectively, compared to the original criteria (74% sensitivity, 86% specificity) [102,126,127]. However, these performance metrics are primarily derived from adult cohorts, which include a broad spectrum of myocarditis phenotypes, and may not be directly extrapolable to the infant population.

Our study focused on a particularly vulnerable subgroup: infants without prior cardiac history who presented with acute heart failure and met the clinical, CMR, and EMB diagnostic criteria for inflammatory cardiomyopathy. Although several paediatric studies have supported the role of CMR in myocarditis, these investigations often suffer from small sample sizes and variability in acquisition protocols across centres [131,132]. Within this cohort, although CMR consistently detected myocardial inflammation in all cases, it did not allow for reliable

differentiation between virus-mediated myocarditis and genetically mediated cardiomyopathies. Our results show that, despite its high sensitivity, the discriminatory power of CMR remains limited when used in isolation in this specific population. The diagnostic yield of CMR is known to vary according to clinical presentation, with higher accuracy in infarct-like myocarditis and lower performance in patients presenting with heart failure or arrhythmic phenotypes [130].

Interestingly, late gadolinium enhancement was the only CMR parameter significantly associated with virus-mediated cases ($p = 0.016$), suggesting its potential utility as a supportive diagnostic clue in these infants. Furthermore, no significant correlation was found between CMR parameters and histological measures of inflammation, underscoring the challenges in interpreting imaging findings.

EMB continues to be considered the *gold standard* for diagnosing myocarditis, as it allows for direct tissue analysis and the identification of infectious agents [4,9,109]. Despite its value, EMB remains underutilized in children due to procedural risks and technical challenges, especially in stable patients without fulminant presentations [1,77]. The focal distribution of myocardial inflammation also raises concerns about sampling error, potentially leading to false-negative results [78].

To improve diagnostic accuracy, the Marburg immunohistochemical criteria were developed, defining myocarditis as ≥14 leukocytes/mm² with at least 7 CD3+ T cells/mm² [107,117]. However, our findings demonstrated that these criteria lack specificity for aetiological differentiation in infants with new-onset heart failure. All patients in our cohort, regardless of underlying cause, fulfilled the Marburg criteria. Moreover, the presence of necrosis or fibrosis on EMB did not distinguish between groups. Interestingly, while all infants showed inflammatory infiltrates, the group without pathogenic genetic variants exhibited significantly higher levels of inflammation and the presence of oedema, correlating with better outcomes. In contrast, infants carrying P/LP variants in cardiomyopathy-associated genes displayed lower levels of inflammation and displayed poorer prognosis, including persistent ventricular dysfunction and progression to heart transplantation.

These results underscore a diagnostic limitation: in the context of acute heart failure in infancy, both CMR and EMB may confirm the presence of myocardial inflammation but fail to clarify its aetiology. This overlap between viral myocarditis and inflammatory phases of genetic

cardiomyopathies suggests that relying solely on imaging or biopsy findings is insufficient for accurate classification.

Therefore, our data support the need for an integrative diagnostic approach that incorporates EMB (not only based on Marburg criteria but also considering the quantitative assessment of inflammation and oedema), detailed CMR analysis (especially LGE distribution), troponin levels, detection of viral genomes within myocardial tissue, and systematic genetic testing. Early and accurate identification of the underlying mechanism remains essential to inform prognosis and guide therapeutic decisions in this high-risk paediatric population.

HYPOTHESIS 5: Ongoing clinical and imaging follow-up is essential for all paediatric patients diagnosed with myocarditis, including those with uncomplicated forms.

To address this hypothesis, we analysed two distinct paediatric cohorts initially diagnosed with myocarditis based on the established criteria of the *European Society of Cardiology* and the *American Heart Association.* The first group included infants with new-onset heart failure, initially diagnosed with myocarditis confirmed by both CMR and EMB. The second cohort comprised adolescents with CMR-confirmed infarct-like (uncomplicated) myocarditis.

In both cohorts, although CMR at presentation was effective in identifying myocardial inflammation, it did not consistently distinguish between virus-mediated forms and cases with an underlying genetic predisposition. However, serial clinical and imaging follow-up proved crucial to uncover hidden genetic susceptibility, detect persistent myocardial abnormalities, and refine individual risk stratification, even among those with an initially uncomplicated presentation.

In the infant cohort, as discussed in previous sections, initial CMR findings using the 2018 Lake Louise Criteria [122] confirmed myocardial inflammation across all patients, irrespective of aetiology. Despite the homogeneity of clinical presentation and imaging findings at baseline, prospective follow-up combined with genetic testing enabled the differentiation of two distinct aetiological groups with significantly different prognoses. Although CMR follow-up was not systematically incorporated into the protocol for this infant group, such surveillance could potentially help identify genetically mediated cases during follow-up, contributing to more accurate prognosis and management.

In the adolescent cohort, all patients met the 2018 Lake Louise Criteria on initial CMR [122] and baseline ejection fraction and LGE burden did not differ significantly between patients with or without P/LP variants either. Only certain imaging features, such as ring-like patterns of LGE or septal involvement, were more frequently observed among gene-positive patients. These patterns have been associated in prior research with adverse outcomes and may reflect early manifestations of inherited cardiomyopathies [49,173,191]. Importantly, CMR at 12-month follow-up revealed clear differences between groups also in those parameters that were indistinguishable at presentation, with gene-positive individuals demonstrating a greater extent of persistent LGE ($p = 0.047$) and ongoing myocardial oedema ($p = 0.009$), suggesting a role of genetic predisposition in sustained myocardial injury and remodelling [49,192].

Although the *European Society of Cardiology* provides a general recommendation for long-term follow-up after an episode of acute myocarditis [4], recent adult national consensus documents suggest that low-risk uncomplicated cases may be discharged to primary care after resolution [137]. However, in our adolescent cohort, several patients who would have been classified as low risk according to these adult criteria were found to carry P/LP variants, underscoring the necessity for prolonged follow-up in the paediatric population, where clear guidelines remain lacking.

Initial imaging findings may assist in early risk stratification. For instance, the *Spanish Society of Cardiology* proposes follow-up strategies based on clinical and CMR features, recommending extended surveillance when LGE is extensive (≥3 segments), involves the interventricular septum, or presents with high-risk patterns such as ring-like distribution [137]. The necessity of repeating EMB or CMR during follow-up remains debated, but in clinical practice, such assessments are justified if ventricular function remains impaired or if biomarkers remain elevated. In cases of apparent clinical resolution, CMR at 6 to 12 months may reveal residual fibrosis or oedema and assist in prognostication [1,9].

The findings of our adolescent cohort support these recommendations, with longitudinal CMR showing that persistent myocardial abnormalities among gene-positive patients, are associated with adverse cardiac remodelling and could indicate an early phase of cardiomyopathy development [49,70,191]. Persistent myocardial inflammation and fibrosis may act as substrates for arrhythmias and ventricular dysfunction, highlighting the importance of ongoing surveillance, especially in younger individuals given their extended life expectancy [191,193].

Our research reinforces the idea that long-term monitoring is advisable not only for complicated myocarditis cases but also for those initially considered low risk. Identifying early signs of genetic cardiomyopathy has major implications for patient care, including sudden death prevention, exercise restriction recommendations, and the implementation of cascade genetic screening in families [49,50]. Further research is required to confirm these observations in other myocarditis phenotypes, for example those presenting with arrhythmias, and to validate these findings in larger, multicentre cohorts, particularly among patients with severe presentations such as fulminant myocarditis or refractory heart failure.

LIMITATIONS

LIMITATIONS

Despite the relevance and novelty of the findings presented in this thesis, some limitations must be acknowledged, inherent to the challenges of investigating an uncommon disease such as paediatric myocarditis.

First, although the study was designed prospectively and involved a multicentre paediatric cohort from several hospitals, myocarditis remains an infrequent condition in childhood. This rarity becomes even more pronounced when analysing specific subgroups, such as infants presenting with complicated myocarditis: acute heart failure or fulminant forms requiring mechanical circulatory support. Consequently, despite representing one of the largest paediatric myocarditis cohorts reported to date, the number of patients in certain subgroups may still be relatively limited, potentially affecting the statistical power of subgroup analyses or limiting the ability to detect significant differences in some variables. This limitation is shared by most published studies in paediatric myocarditis, where the heterogeneity of clinical presentations and the low incidence of the disease complicate the development of robust prospective cohorts.

Second, although the diagnostic criteria applied were rigorous and based on the current guidelines of the *European Society of Cardiology (ESC)* [4] and the 2021 *American Heart Association (AHA)* Scientific Statement on myocarditis in children [9], the diagnosis in many patients was based on clinical presentation and CMR findings. The gold standard for a diagnosis remains the EMB, which was not performed systematically due to its invasive nature, particularly in stable patients with uncomplicated myocarditis, where the expected clinical course is favourable, and the risk-benefit balance does not justify its routine use. Consequently, the absence of histological confirmation in a proportion of cases could limit the definitive aetiological classification.

Third, microbiological testing was performed using blood PCR analysis in most patients, whereas myocardial tissue PCR was only available in those undergoing EMB. Although our results (as detailed in Article 4) suggested a good correlation between blood and myocardial PCR, particularly in younger patients, this approach may have influenced the sensitivity of viral detection and potentially underestimated viral replication in the myocardium for some patients. Conversely, as reported in adult cohorts, the presence of viral genome in myocardial tissue has

been associated with latent persistence without active disease or causality, raising additional uncertainty regarding the interpretation of viral PCR results [35,167].

Fourth, although CMR findings were carefully analysed and the application of the 2018 Lake Louise criteria ensures high sensitivity for detecting myocardial inflammation [128], our results confirmed the limited capacity of CMR alone to reliably differentiate virus-mediated myocarditis from early-stage genetic cardiomyopathies, particularly in infants with new-onset heart failure, as shown in Articles 1 and 3. This reinforces the need for integrating additional diagnostic tools, including genetic testing and histopathological studies, to improve aetiological accuracy. Given this context, and acknowledging the inherent limitations of current diagnostic tools, inclusion criteria for the present study required the fulfilment of clinical and CMR-based diagnostic criteria for myocarditis, as established by international scientific societies. In addition, for those patients who underwent EMB, histological confirmation of myocarditis was mandatory, based on the Marburg immunohistological criteria [107,117].

Fifth, with regard to genetic analysis, although this study applied comprehensive NGS techniques in patients with suspected genetic background, current genetic testing methods have intrinsic limitations. Even in patients with cardiomyopathies of established genetic origin, such as HCM or ARVC, the detection rates remain incomplete. For example, the reported diagnostic yield in isolated DCM is approximately 15–25%, increasing in familial or transplanted cases; around 30% in sporadic HCM (up to 60% in familial forms), and up to 50% in ARVC and NDLVC [138]. Therefore, a negative genetic result does not exclude a genetic substrate in some patients, especially considering the current limitations in variant interpretation and the existence of yet unknown disease-causing genes.

Finally, although the follow-up period was appropriate for a paediatric population in most of the cases, and covering detailed clinical, imaging, and genetic evaluation, it might still be insufficient to detect late-onset complications or the development of overt cardiomyopathy in genetically predisposed patients, as some inherited cardiomyopathies may develop progressively over years or even decades. Thus, long-term follow-up into adulthood will be highly recommended to fully understand the prognostic implications of the findings reported in this thesis and to confirm the potential risk of progression in certain subgroups.

CONCLUSIONS

CONCLUSIONS

This doctoral thesis provides novel insights into the clinical presentation, aetiological spectrum, and prognostic factors of acute myocarditis in the paediatric population, with a particular focus on the role of early diagnostic tools in guiding clinical decision-making and improving patient outcomes. The conclusions derived from this work are the following:

1. **Clinical heterogeneity and early prognostic indicators.**

Acute myocarditis in childhood is a highly heterogeneous condition, ranging from mild presentations to fulminant forms. This work confirms that, despite advances in diagnostic methods, the initial clinical presentation remains insufficient to accurately predict the underlying cause or long-term prognosis in many cases, both in complicated forms (with heart failure or fulminant myocarditis) and in uncomplicated forms.

2. **The role of PVB19 infections and the diagnostic value of ECG patterns.**

Standard diagnostic tools, such as the 12-lead electrocardiogram, may offer valuable, early clues to the viral aetiology of myocarditis in cases of PVB19 infection. In this context, a specific pattern characterised by peaked P waves, low QRS voltages, and repolarization abnormalities (flattened or inverted T waves, with or without QTc prolongation) has demonstrated a high specificity and moderate sensitivity. Nevertheless, electrocardiographic and CMR findings alone lack sufficient discriminatory power to identify those patients with an underlying genetic substrate or to predict long-term prognosis. Therefore, a multimodal diagnostic approach is needed.

3. **Genetic variants in uncomplicated myocarditis.**

A significant proportion of paediatric patients with uncomplicated myocarditis carry P/LP variants in cardiomyopathy-associated genes. Notably, these variants can be identified in patients without family history, highlighting the silent and often unexpected genetic substrate underlying paediatric myocarditis.

Genetic testing should be considered in all paediatric patients with clinically suspected myocarditis, with particular emphasis on those presenting with new-onset heart failure or

fulminant forms, especially when response to standard heart failure treatment is suboptimal. Additionally, genetic evaluation should also be performed in patients with initially uncomplicated myocarditis when specific red flags are present. These include a positive family history of cardiomyopathy, recurrent episodes of myocarditis, or the presence of characteristic CMR findings (such as LGE ring-like pattern or septal involvement), as well as persistent myocardial oedema on T2-STIR sequences or extensive LGE persistence on follow-up imaging

4. CMR and EMB in guiding diagnosis.

Although CMR and EMB at presentation are effective in identifying myocardial inflammation, they do not consistently distinguish a potential genetic background. In this prospective cohort, myocardial inflammation was universally observed, regardless of the underlying aetiology. This highlights the current limitations of both EMB, even when using the Marburg criteria, and CMR imaging, applying the 2018 Lake Louise criteria, in clearly differentiating between virus-mediated myocarditis and genetic cardiomyopathies. While CMR is highly sensitive for detecting myocardial inflammation, its specificity in determining the aetiology appears limited at presentation. Therefore, a comprehensive diagnostic approach is necessary, integrating EMB findings (particularly T-lymphocyte count and the presence of oedema), CMR parameters (LGE distribution and mapping values), detection of viral genome in myocardial tissue, biochemical markers such as troponins, and genetic testing, to guide accurate diagnosis and treatment.

5. Systematic genetic testing within a multimodal diagnostic framework.

The findings of this thesis support a paradigm shift that is gradually emerging in the approach to paediatric myocarditis. Moving beyond the traditional view of myocarditis as an exclusively acquired and self-limiting disease, this research highlights the interplay between environmental triggers, immune-mediated injury, and genetic predisposition. A personalised approach, integrating clinical presentation, EMB, CMR, microbiological studies and genetic profiling, is essential to guide prognosis, optimise therapeutic strategies, and improve long-term outcomes in these patients.

The use of genetic testing in selected clinical scenarios, represents a key tool in the aetiological evaluation of myocarditis. Its integration into clinical practice facilitates not only accurate

diagnosis but also enables family screening strategies, guides sports and lifestyle recommendations, and allows for appropriate arrhythmic risk stratification.

6. The importance of structured long-term follow-up.

This work reinforces the importance of longitudinal follow-up in all paediatric patients diagnosed with myocarditis, including those initially presenting with mild and uncomplicated forms. Serial clinical evaluations combined with advanced imaging studies allow the detection of persistent myocardial injury or the progression to ventricular dysfunction and features suggestive of a genetic background.

Future research should aim to validate these findings in larger, prospective, multicentric paediatric cohorts and to explore novel biomarkers that may improve early aetiological differentiation. Additionally, long-term follow-up studies extending into adulthood will be necessary to fully characterise the natural history of paediatric myocarditis and to understand the prognostic implications of the genetic findings reported in this thesis.

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