4 Etiology and Pathogenesis of Fulminant Myocarditis

Chen Chen and Dao Wen Wang

4.1 Etiology of Fulminant Myocarditis

The etiology of fulminant myocarditis (FM) is similar to that of acute and non-fulminant myocarditis, which includes infectious and non-infectious factors (Table 4.1).

4.1.1 Infectious Factors

Viral infection is the main cause of myocarditis; 1–5% patients with acute viral infection may have symptoms of myocarditis [1]. However, due to the limitations of detection technology and difficulties in the acquisition of samples, only 10–20% patients with viral myocarditis test positive on the myocardial tissue virology test. In a prospective clinical study containing more than 670,000 Finnish men, a total of 98 patients were clinically diagnosed with myocarditis during a 20-year period, and only 4% tested positive for coxsackievirus [1].

In the 1980s and 1990s, it was difficult to isolate and culture infected virus strains from the heart tissue of patients with myocarditis. Through serological examination, enterovirus (including coxsackievirus) and adenovirus have been associated with myocarditis based on the co-occurrence of increased virus titers and acute heart failure performance [2]. With the rapid development of molecular biology techniques, the detection methods of various viruses have constantly improved. Case reports and series have associated myocarditis with more than 20 viruses, including parvovirus and human herpesvirus (HHV) (Fig. 4.1) [3]. Some studies have indicated that enterovirus was the only virus type detected in the hearts of French patients with myocarditis, while several studies have shown that mostly parvovirus B19 (PVB19) and human herpesvirus type 6 (HHV6) only are detected in the hearts of German patients, and coxsackievirus is rarely found [4, 5]. In the United States, adenovirus and enterovirus were the most common pathogenic viruses reported in 2003, while parvovirus was the major pathogenic virus reported in 2010 [6, 7].Currently, it is unclear whether the etiology of myocarditis is regional or epidemic; these differences in the detected virus types may be caused by the diverse epidemic spectrum, the non-specificity of primers and antibodies used for virus detection, different detection schemes, and the limited sample size [3]. Currently, the Center for Disease Control and Prevention (CDC) in several countries is observing the correlation between the outbreak cycle and the region of viral

C. Chen (✉) · D. W. Wang (✉)
Division of Cardiology, Department of Internal Medicine and Hubei Key Laboratory of Genetics and Molecular Mechanisms of Cardiological Disorders, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China
e-mail: chenchen@tjh.tjmu.edu.cn; dwwang@tjh.tjmu.edu.cn

© The Author(s), under exclusive license to Springer Nature Singapore Pte Ltd. 2022
D. W. Wang (ed.), *Fulminant Myocarditis*, https://doi.org/10.1007/978-981-19-5759-8_4

Table 4.1 Possible etiology of myocarditis

Infectious factors	
Viruses	Adenovirus, enteroviruses (such as coxsackievirus and poliovirus), arboviruses, cytomegaloviruses, dengue viruses, echoviruses, Epstein-Barr virus, hepatitis C virus, herpes virus, human immunodeficiency virus, influenza virus, coronavirus, mumps virus, parvovirus, rabies virus, rubella virus, varicella virus, varicella zoster virus, hemorrhagic fever virus, yellow fever virus
Bacteria	Brucella, cholera, *clostridium*, *Corynebacterium diphtheriae*, *haemophilus*, *legionella*, *meningococcus*, *Neisseria gonorrhoeae*, *salmonella*, *staphylococcus*, *clostridium tetani*, *tuberculosis*, *Francisella tularensis*
Spirochete	*Leptospira*, Lyme disease spirochete, relapsing fever spirochete, *treponema pallidum*
Fungi	*Actinomycetes*, *aspergillus*, *Blastomyces, Candida*, *Coccidioides*, *cryptococcus*, *histoplasma*, *mucor*, *nocardia*, *Sporothrix*
Rickettsia	Rickettsia burneti, Rickettsiatyphi, rickettsia Prowazeki, rickettsia Mooseri
Protozoa	Trypanosoma, Ameba, Trypanosoma cruzi, leishmania spp., plasmodial, toxoplasma gondii
Helminth	*Ascariasis*, *echinococcosis*, filariasis, *paragonimiasis*, *schistosomiasis*, *strongyloidiasis*, *trichinosis*
Other	*Mycoplasma*
Noninfectious factors	
Systemic diseases	Celiac disease, connective tissue disease, Wegener granuloma disease, Kawasaki disease, eosinophilia, sarcoidosis, thyrotoxicosis
Hypersensitivity	Antibiotics, clozapine, diuretics, insect bites, lithium, snakebite, tetanus toxoid, mesalazine
Cardiotoxic substance	Alcohol, anthracyclines, arsenic, carbon monoxide, catecholamines, cocaine, heavy metals

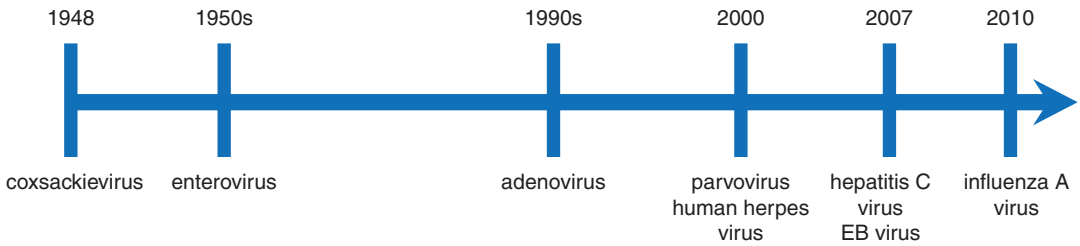

Fig. 4.1 Evolution of major viral causes of myocarditis over time

myocarditis and other viral diseases. In 2006, a Japanese retrospective study found that the detection rate of hepatitis C virus (HCV) in the hearts of 1355 patients with myocarditis was 4.4% [8]. In 2010, influenza A virus was detected for the first time in the hearts of patients with viral myocarditis in the United States at a rate of approximately 10%; the rate in the hearts of patients with FM was approximately 5% [9]. A recent study used nested polymerase chain reaction (PCR) to detect the virus genome (including enterovirus, PVB19, adenovirus, cytomegalovirus, Epstein-Barr virus, and HHV6) in the myocardium of 27 patients with FM from 16 medical centers in the United States, Europe, and Japan. Consequently, PVB19 was only detected in five (18.5%) samples [10]. Recently, Heidecker et al. examined peripheral blood and myocardial tissue samples from 33 patients with myocarditis using virome capture sequencing and identified the following viruses: EB virus, hepatitis G virus, human endogenous retrovirus K, and anaerobic virus. Among them, human endogenous retrovirus K was detected in all the blood and tissue samples from two FM and 13 giant cell myocarditis samples [11]. Cases of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-induced FM have also been reported in the recent outbreak of the coronavirus disease (COVID-19) pandemic [12].

These studies have provided partial evidence for the etiology and epidemiological characteristics of viral myocarditis, suggesting that the clinical symptoms of myocarditis caused by different virus types may be heterogeneous. It is important to enhance the understanding of virology, immunology, pathology, and clinical medicine in myocarditis.

4.1.1.1 Enterovirus

Enterovirus, especially coxsackievirus B3 (CVB3), is the primary pathogen of myocarditis. Enteroviruses belong to Picornaviridae, a family of single-strand RNA viruses containing 10 enteroviruses and three rhinoviruses (Fig. 4.2). These viruses are widely distributed and highly pathogenic; they are the causative pathogens of several serious diseases widely prevalent in vertebrates, including humans. Infection with these viruses can lead to either temporary organ dysfunction, persistent irreversible organ function damage, or even death. Enteroviruses are the causes of severe diseases, such as polio, aseptic meningitis, enteroviral encephalitis, and enteroviral vesicular stomatitis, as well as the common cold. Enteroviruses can spread easily from person to person through airway and fecal-oral routes, and infection can also occur by touching items contaminated with enteroviruses. The peak incidence of enterovirus infection occurs mostly in summer. Due to the long asymptomatic incubation period for enterovirus, outbreaks can occur suddenly and are difficult to prevent. Currently, there is no effective drug specific for enterovirus, and treatment is still focused on symptomatic support and symptom control [13].

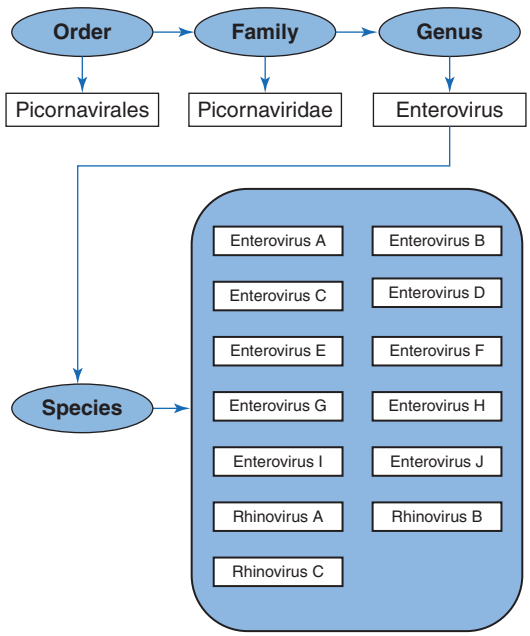

Fig. 4.2 Virological classification of enteroviruses

1. Etiological characteristics: Enterovirus viruses have a single-stranded RNA genome, 15–30 nm icosahedral spherical capsid, and no envelope. When a virus infects a host cell, it first binds to receptors on the cell surface (mainly integrins and immunoglobulin-like proteins) and penetrates the cell membrane. When the virus enters the host cell, viral RNA molecules are released from the capsid to synthesize viral proteins and promote viral replication, which ultimately leads to the death of the host cell. Subsequently, the virus is released from the cytoplasm and can continue to infect other cells [14].

   Enterovirus has a highly recessive infection rate and strong resistance to physical and chemical factors. It can survive for several days at room temperature and can be preserved for a long time at −20 °C. Enterovirus is resistant to ether, acid (pH 3–5), and bile. It can survive in sewage and feces for several weeks, but it is sensitive to heat, drying, and ultraviolet light. It can be inactivated after 30 minutes of treatment at 56 °C. Various oxidants such as potassium permanganate and hydrogen peroxide solution (hydrogen peroxide) can play a role in disinfection. Enterovirus can replicate rapidly and cause pathophysiological changes in host cells within 2–7 days after colonization in suitable host cells.
2. Epidemiology Enteroviruses are transmitted via the fecal–oral route or the respiratory tract. Once infected, enteroviruses can continuously exist in respiratory secretions and feces of patients for 1–3 and 2–8 weeks, respectively.

Enteroviruses are widespread globally. A total of 71 serotypes of enteroviruses have been identified using neutralizing antibodies. The main virus serotypes circulating in various regions are constantly changing, and infection rates far exceed the number of clinically diagnosed cases. Data provided by the National Enterovirus Surveillance System of the CDC showed that from 1970 to 2005, 15 representative enterovirus serotypes accounted for 83.5% of all isolated enterovirus strains provided by public health laboratories at all levels in the U.S. The number of infected cases increases sharply in summer and early autumn and usually reaches its peak in August [15].

4.1.1.2 Adenovirus

Adenoviruses are double-stranded DNA viruses that generally cause mild-to-moderate respiratory and/or gastrointestinal tract infections. They can also induce hemorrhagic cystitis, hepatitis, hemorrhagic colitis, pancreatitis, nephritis, or meningoencephalitis in rare cases. Epidemic adenovirus infection is more likely to occur in children or relatively closed populations, such as individuals in the military. Children are more susceptible to adenovirus because of their underdeveloped humoral immunity. Immunocompromised populations (such as patients undergoing organ transplant and people with HIV) are more susceptible to adenovirus and tend to have more severe symptoms. The mortality rate of severe pneumonia or other organ damage caused by adenoviruses exceeds 50%. At present, more than 50 different serotypes of adenovirus have been discovered. The tissue susceptibility and clinical symptoms induced by different adenovirus serotypes are not entirely similar. The serotypes prevalent in different periods may differ, and the serotypes prevalent in different countries and regions may also vary in the same period. Serotypes 2 and 5 tend to invade the myocardium and cause myocarditis. Due to the lack of prospective randomized controlled clinical trials, the specific treatment regimen for adenovirus infection is controversial. Cidofovir is recommended for the treatment of patients with severe adenovirus infection, but it is not suitable for all patients. The live oral vaccine, which is effective in reducing respiratory adenovirus infections, is routinely used in the US military, but it has not been popularized in the general population [16].

1. **Etiological Characteristics** Human adenoviruses are a group of non-enveloped double-stranded DNA viruses belonging to the mammalian adenoviruses of Adenoviridae. They are icosahedral, 10–100 nm in diameter, and contain seven viruses (HADV-A to HadV-G), the first six of which have caused global outbreaks of human epidemic infections. Fifty-one serotypes (numbered 1–51) have been identified, and more than 70 adenovirus genotypes (including those numbered 52, 53, 54) have been predicted by bioinformatics comparison. Nearly 20 serotypes of adenovirus are pathogenic in humans. Patients and persons with recessive infection are the primary sources of adenovirus, which can be transmitted through the respiratory tract, the fecal–oral route, and contact with contaminated tissues or the blood. The latent period varies among different serotype-induced infections. Adenoviruses can resist various disinfectants, but they are sensitive to 95% ethanol [17].
2. **Epidemiology** Adenoviruses cause epidemics of respiratory diseases, conjunctival pharyngeal fever, keratitis, and gastrointestinal diseases, with a self-limited course in most patients. Severe adenovirus infection occurs most frequently in immunocompromised patients and is rare in individuals with normal immunity. Adenovirus infection can occur round the year without apparent seasonality, but the epidemic period is mostly in winter or early spring. The main route of infection is contact with exposed people and infected objects, including respiratory transmission, conjunctival contact, and the fecal–oral route. The incubation period for adenovirus ranges from 2 days to 2 weeks, and asymptomatic adenovirus carriers can carry the virus for months. Importantly adenoviruses can be dormant for years in other tissues, such as lymphoid tissue and renal parenchyma, and can be reactivated when the host is severely immunosuppressed. Adenovirus can easily spread in closed environments, such as hospi-

tals, public swimming pools, childcare institutions, boarding schools, and long-term care centers [18].

4.1.1.3 Parvovirus

Parvovirus is a non-enveloped virus with a diameter of less than 25 nm and has a linear single-stranded DNA genome of 5–6 kb with hairpins at both ends. Adeno-associated virus (AAV) was the first parvovirus discovered to infect humans, but it is not pathogenic. Subsequently, two kinds of pathogenic parvovirus were discovered—human PVB19 and human Bocavirus (HBoV) 1. PVB19 is highly pathogenic and can induce a series of diseases, including infectious erythema, regenerative disorder crisis in patients with chronic hemolytic anemia, chronic anemia in patients with immunosuppression, pregnancy abortion, stillbirth, and joint disease. Patients with latent infection can carry the virus for a long time without any symptoms. Studies have reported that PVB19 infection is closely associated with acute and chronic myocarditis, and patients with partial dilated cardiomyopathy have higher PVB19 virus titer in the myocardium than healthy person [19]. Other researchers have observed increased neovascularization around inflammatory cells in the hearts of patients with FM caused by PVB19 [20]. HBoV1 infection is an important cause of acute respiratory tract infection, and wheezing is the most common symptom of HBoV1 infection. The clinical significance of other parvovirus infections such as parvovirus 4 (PARV4), HBoV2, HBoV3, and HBoV4 is unclear. Currently, there is no vaccine or specific antiviral drug for parvovirus [21].

1. **Etiological characteristics:** The capsid of parvovirus has icosahedral symmetry with two capsid proteins, VP1 and VP2, of which the is located outside the shell and easily binds to antibodies. In general, PVB19 is particularly cytotropic to human erythrocytes and can grow in fresh human bone marrow cells, peripheral blood cells, fetal liver cells, erythroleukemia cells, and umbilical cord blood cells. However, PVB19 can invade endothelial cells in myocarditis and dilated cardiomyopathy. PVB19 is heat resistant and can survive for 30 minutes at 56 °C [22].
2. **Epidemiology:** Outbreaks of PVB19 infection occur mainly in winter and spring. PVB19 infection is widespread worldwide and its epidemic pattern is regional. Half of all adults have been infected with PVB19. The positive rate of PVB19 antibodies in the population increases with age—2%–20% in children aged under 5 years, 15–40% in adolescents aged 5–18 years, and 40–80% in the adult population. The virus neutralizing immunoglobulin (Ig) G is produced by organisms 2 weeks after PVB19 infection, which can effectively remove the virus from the blood and ensure lifelong resistance by inducing immune response [23–25].

PVB19 is primarily transmitted via the respiratory route, but the prodromal symptoms are mainly fever, fatigue, headache, and myalgia rather than respiratory symptoms. It is unclear how PVB19 travels through the airway epithelial barrier to the bone marrow. PVB19 can also be transmitted through the blood or from mother to child. PVB19 virus DNA can be detected in the airway when prodromal symptoms occur, suggesting a high viral titer in the patient. However, the severity of the acute phase is unrelated to viral load. With remission, the virus titer decreases rapidly and can persist for months, even years.

4.1.1.4 Human Herpes Virus

There are eight species of human herpes viruses (HHV), all belonging to Herbviridae, which can be divided into three subfamilies—α, β, and γ—by genetic analysis of their conserved structural protein gH (Table 4.2) [26]. Herpes viruses of α subfamily have a wide range of hosts. They are a kind of cytolytic viruses with a short replication cycle and fast reproduction rate, mostly lurking in sensory ganglia. The host range of β subfamily herpesviruses is relatively narrow. The infected cells grow and form giant cells. The virus can cause latent infection in lymphocytes as well as secretory glands, the kidneys, or other tissues. γ subfamily herpetic viruses mainly infect B lymphocytes and remain latent for a long time, most of which do not cause cytolytic diseases.

HHV infection can be latent for a long time and cause damage when host immunity is decreased.

Table 4.2 Classification of HHV

Subfamily	Virus	Abbreviation	Adult Infection rate
α	Herpes simplex virus 1	HSV-1	Approx 70%
α	Herpes simplex virus 2	HSV-2	Approx 30%
α	Varicella-zoster virus	VZV (HHV-3)	>95%
γ	EB virus	EBV (HHV-4)	Approx 85%
β	Human cytomegalovirus	HCMV (HHV-5)	Approx 70%
β	Human herpesvirus 6A	HHV-6A	Approx 95%
β	Human herpesvirus 6B	HHV-6B	?
β	Human herpesvirus 7	HHV-7	Approx 85%
γ	Kaposi sarcoma-associated virus	KSHV (HHV-8)	?

Note:? Unknown. approx, approximately

Seventy percent adults have been infected with HSV-1, which usually causes fever and occasionally severe encephalitis. Thirty percent adults have been infected with HSV-2, which usually causes genital herpes and occasionally severe neonatal infections. Almost all adults have been infected with VZV, which causes chickenpox and shingles, and EBV, which is the leading cause of infectious mononucleosis. EB virus can also cause Burkitt lymphoma and nasopharyngeal cancer.

HCMV is also a pathogen that cause infectious mononucleosis, and HCMV infection is an important cause of congenital deafness and intellectual disability. Persistent HCMV infection is also associated with cardiovascular diseases such as coronary heart disease. HHV-6 is a pathogen that cause myocarditis. The pathogenicity of HHV-7 has not been determined, and it may be associated with drug eruption.

Both KSHV and EBV belong to the γ subfamily. KSHV mainly infects lymphocytes of immunodeficient patients, leading to malignant diseases such as Kaposi sarcoma. It is currently the only confirmed carcinogenic human herpes virus. Recently, non-coding RNA expression profiling revealed that KSHV may also be involved in the occurrence and development of myocarditis. KSHV can encode microRNA (miRNA) to increase the susceptibility of model animals to CVB3 infection by inhibiting the body's own defense mechanisms.

1. **Etiological characteristics** Mature virions are approximately 200 nm in diameter. All herpes viruses are composed of three main structures:
   1. Spherical icosahedral stereosymmetric nucleocapsid with a diameter of 90–110 nm and a linear double-stranded DNA genome
   2. The outermost layer is the capsule with glycoprotein spikes
   3. The nucleocapsid and capsule are filled with a protein mixture

   HHV-6 has serological and genetic characteristics that differ from other herpes viruses. Its genomic DNA ranges from 160 to 170 kb, and it can be divided into two types, HHV-6A and HHV-6B, according to its antigenicity. The two types of HHV-6 have similar heritability, but different epidemiological and clinical characteristics [27]. The pathogenicity of HHV-6A is unknown, and HHV-6B can cause herpes and myocarditis in children [28].
2. **Epidemiology**: HHV-6 is widely prevalent worldwide. Most adults in Europe and America have been infected with HHV-6 [29, 30]. It is now believed that HHV-6 infection rates continue to increase between 6 and 18 months after birth due to the gradual depletion of antibodies from the mother, and then slowly decline with age.

   HHV-6 nucleic acid can often be detected in the saliva, suggesting that HHV-6 can be latent in salivary glands. Therefore, the saliva could work as a vehicle for fecal–oral infection.

4.1.2 Non-infectious Factors

Anti-tumor drug-induced myocarditis, especially FM, should not be ignored [31]. In the past 20 years, new approaches to cancer treatment have proliferated, leading to dramatic improvements in the prognosis of some cancers. However, both traditional anti-tumor drugs and various new tumor drugs can cause cardiovascular toxicity, including myocarditis [32]. Anthracyclines have been previously found to cause cardiotoxic effects such as myocarditis–pericarditis syndrome [33]. Recently, myocardial injury, especially myocarditis, caused by novel anti-tumor drugs such as immune checkpoint inhibitors (ICIs) has attracted increasing attention [34]. ICIs are antibody-targeting immune checkpoints that eliminate tumors by inhibiting the immune escape of tumor cells and enhance the immune response of T cells. It is a milestone of progress in tumor therapy in recent years and has greatly improved the prognosis of some patients with cancer. As of 2019, at least seven ICIs have been approved for marketing and many more are in development [35]. However, ICIs can also cause several immune-related adverse reactions, including colitis, dermatitis, pneumonia, and myocarditis [36].

In 2016, Johnson et al. reported two patients with ICI treatment-induced FM for the first time. Both patients presented with malignant arrhythmias and myocarditis, and pathological results suggested massive T cells and macrophages infiltrated in the myocardium [37]. According to statistics, the incidence of myocarditis caused by ICIs is approximately 1%, while programmed cell death protein 1 (PD1) and programmed cell death ligand 1 (PDL1) causes a higher incidence of myocarditis than cytotoxic T lymphocyte antigen 4 (CTLA4) (Table 4.3) [38–41]. A recent study including 101 cases of ICI-induced myocarditis showed that its incidence increased annually. Moreover, the combined use of multiple ICIs significantly increases the incidence of myocarditis, with an average onset time of 27 days after ICI use, while the mortality rate is as high as 46% [42]. Fifty-seven percent patients in the study received anti-PD1 therapy and 27% received anti-CTLA4 combined with anti-PD1 or anti-PDL1 therapy. Among them, 59 patients had detailed medication records; 76% patients developed the disease within 6 weeks of initiating the medication (5–155 days) and 64% patients developed the disease after only one or two doses of medication.

FM accounts for approximately 15% of ICI-induced myocarditis, and the higher the troponin level, the worse the prognosis. Some patients respond well to glucocorticoid treatment [39].

The etiology of FM varies, and the histological appearance of FM caused by different etiologies has a certain tendency. For example, FM caused by various viruses and ICIs is more likely to present as lymphocytic myocarditis; eosinophilic myocarditis is often caused or accompanied by autoimmune diseases. There are also some differences in the treatment of FM caused by different etiologies. Therefore, it is helpful to clarify the etiological diagnosis based on clinical diagnosis for formulating better treatment strategies and improving patient prognosis.

4.2 Pathogenesis of FM

FM is a cardiac inflammatory process that manifests as rapid cardiac functional collapse and even acute heart failure. The underlying pathogenesis of FM is unclear. The lack of comprehensive acknowledgment regarding the pathophysiological mechanisms behind FM has

Table 4.3 ICIs that can cause myocarditis

ICIs	Category	Disease	References
Ipilimumab + navumab	Anti-ctla-4 + anti-PD-1	Melanoma	[37, 43, 44]
Ipilimumab	Anti-CTLA-4	Melanoma	[45]
Pembrolizumab	Anti-PD-1	Melanoma	[46]
Navumab	Anti-PD-1	Melanoma	[47]
Navumab	Anti-PD-1	Non-small cell lung cancer	[48, 49]

largely hindered the development of effective treatment regimens. Previous research has indicated that immune dysfunction and formation of cytokine storms may be the key pathogenesis of FM (Fig. 4.3), but further research is required to elaborately elucidate the detailed mechanisms.

Cytokine storm syndrome (CSS), also named cytokine storm, is a drastic immune attack from abnormally activated immune cells and cytokines on the human body. CSS is not a specific disease, but is a collective name of a pathophysiological phenomenon in different conditions. In rheumatological diseases, such as systemic juvenile idiopathic arthritis (SJIA) and systemic lupus erythematosus, CSS is commonly called macrophage activation syndrome [50]. Immune checkpoint inhibitors and CAR-T-induced CSS is called cytokine release syndrome (CRS) [51]. In inflammatory diseases such as sepsis and severe acute respiratory syndrome coronavirus (SARS-CoV)- or SARS-CoV-2-induced severe pneumonia, the concept of CSS and CRS have commonly been used interchangeably. Generally, CSS that occurs in the pathogenesis of FM is directly called a cytokine or inflammatory storm. The characteristic of CSS is overwhelming inflammation induced by the positive feedback between over-activated immune cells and inflammatory cytokines. The fierce attack of the immune system against pathogens or damaged cells could simultaneously cause extensive self-tissue damage, leading to multiple organ damage and even endanger patients' lives.

Clinically, the diagnosis of CSS primarily relies on the detection of increased cytokines in peripheral blood. Due to diverse etiology and pathogenesis of CSS, the cytokine spectrum varies in different diseases [52] (Table 4.4).

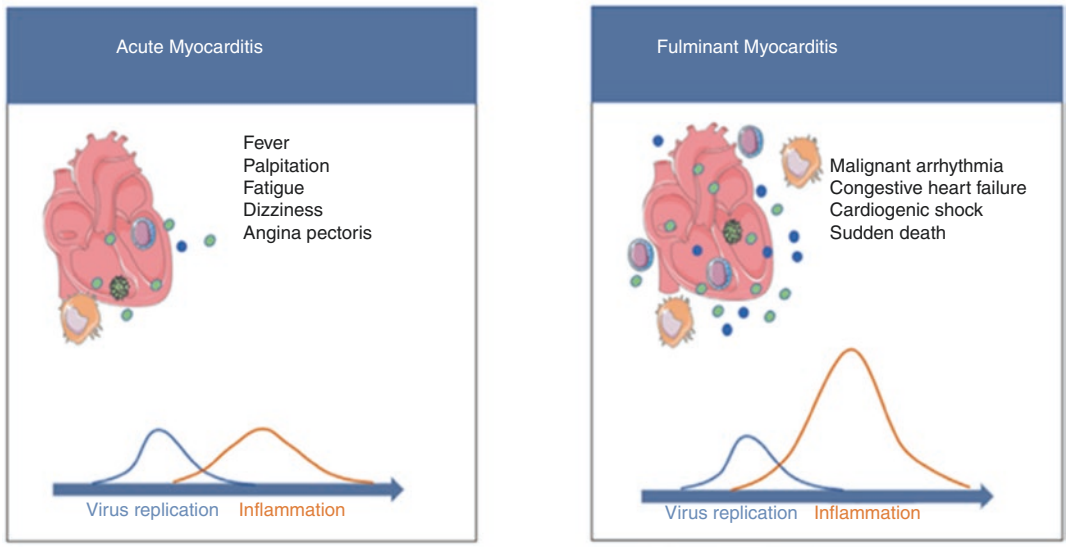

Fig. 4.3 Over-activated immune response and formation of cytokine storms are the main differences between the pathogenesis of FM and AM

Table 4.4 Major cytokines involved in CSS triggered by different etiologies

Etiology	Major cytokines	Reference
CVB3	IL-1β, IL-2, IL-6, TNF-α, IL-1Ra, sTNFR-1, IL-10, IFNs, IL-4, IL-17B	[53, 54]
CAR-T	IFN-γ, IL-2, IL-2Ra, IL-6, sIL-6R, IL-1, IL-10, GM-CSF, IL-12, TNF-α, IFN-α, MCP-1, MIP-1A	[55, 56]
SARS-CoV	IL-1β, IL-6, IL-12, IFN-γ, IP10, MCP-1	[57–59]
MERS-CoV	IFN-γ, TNF-α, IL-15, IL-17	[57, 59]
H1N1	IL-8, IL-9, IL-17, IL-6, TNF-α, IL-12p70 IL-15, IL-6	[60]
Macrophage activation syndrome	IL-1β, IL-6, IL18, TNF-α, IFN-γ	[50, 61]

The roles of the major cytokines in the cytokine storm process of FM are discussed in Chap. 5.

4.3 Laboratory Test

The main laboratory test for FM includes pathogenic detection and cytokine detection.

Pathogenic detection Although both direct cytopathic effects of the pathogen and immune response-mediated myocardial injury were considered to explain the pathogenesis of myocarditis, the detailed mechanisms varied among different pathogens [62–64]. Thus, pathogenic detection may aid in optimizing therapeutic regimens.

4.3.1 Enterovirus

The enterovirus genome is single plus-stranded RNA, which can be used as mRNA to guide viral protein translation. The genome consists of approximately 7500 nucleotides, including (1) the 5′ untranslated region (UTR) length of approximately 750 nucleotides, which forms a secondary structure of RNA and is used to regulate viral replication and translation; (2) an open reading frame length of approximately 6700 nucleotides that encodes a polyprotein; and (3) the 3′ UTR length of approximately 70–100 nucleotides that is used to regulate virus replication (Fig. 4.4). Four structural proteins (VP1, VP2, VP3, and VP4) and seven non-structural proteins (2A, 2B, 2C, 3A, 3B, 3C, and 3D) were cleaved from the multiple proteins encoded by the open reading frame. Among them, 2C, 3C, and 3D proteins are the most conserved in evolution, while 2A, 2B, and 3A usually have high variability and often have different sources in different viruses [65].

The reported detection rate of enterovirus in endocardial biopsy samples of patients with myocarditis is 3–53% [66]. According to the characteristics of the enterovirus genome, the following three detection methods are currently used to diagnose enterovirus infection (Table 4.5).

1. **Nucleic acid detection**: PCR is the most sensitive method for detecting enterovirus nucleic acid in cerebrospinal fluid samples. For samples from cerebrospinal fluid and respiratory secretions, PCR is much more sensitive to enterovirus detection (86%) than virus cultures (30%). Currently, four commercial multiplex PCR kits are available for the detection of enterovirus in nasopharyngeal swab samples. However, the detection of enterovirus by PCR is not practical since stool samples may contain substances that inhibit the PCR reaction.
2. **Virus isolation and culture**: The cerebrospinal fluid, pericardial effusion, the peripheral blood, feces, and various tissues from enterovirus-infected patients could be collected for culture. Enterovirus strains could be isolated from the culture after 2–5 days. After isolation, serotypes of enterovirus can be identified by RNA sequencing. The detection rate of enterovirus can be improved by multi-sampling.

Table 4.5 Detection methods for enterovirus

Detection method	Detection time	Sensitivity	Specificity
Nucleic acid test	1–2 h	100%	97%
Virus culture	3–8 days	80%	100%
Serological test	Several weeks	Limited application	Limited application

Fig. 4.4 Schematic diagram of the enterovirus genome

3. **Serological testing**: Serological testing is limited in acute enterovirus infection due to the following reasons:
   The of antibody titer differs between the acute and convalescent stage
   Cross-reactions may occur between different serotypes
   A lack of highly sensitive IgM assays

The microneutralization method is generally used to detect anti-enterovirus antibodies in patients. However, due to its poor sensitivity, low standardization, and time-consuming characteristics, its application in the routine diagnosis of enterovirus infection is limited.

4.3.2 Adenovirus

The reported detection rate of adenovirus in endocardial biopsy samples of patients with myocarditis is 2–20% [67]. Generally, adenovirus can be detected by immunohistochemistry/fluorescence staining, virus culture, and PCR using samples collected from infected sites (such as nasopharyngeal secretions, pharyngeal swabs, bronchoalveolar lavage, urine, feces, and blood) [68].

1. **Nucleic acid test**: Currently, PCR is the most commonly used method in the clinical diagnosis of adenovirus infection. It is applicable to various clinical samples such as the plasma and urine and is highly sensitive. PCR can also be used to quantify the adenovirus titer and evaluate the therapeutic effect. Some studies have suggested that regular adenovirus detection in the blood and stool samples of high-risk organ transplant recipients can predict adenovirus infection and enable early treatment, but it is unclear whether it should be widely used in patients undergoing organ transplant. Molecular typing of adenovirus by PCR is helpful for the analysis of adenovirus epidemic strains, but since there is no significant difference in the clinical treatment plan for each adenovirus type, detection has not been routinely conducted in clinics.
2. **Virus isolation and culture**: Virus culture is the golden standard for the detection of adenovirus infection, but it is not sensitive to blood samples and may take up to 21 days to detect.
3. **Serological testing**: Serological testing of adenovirus using neutralizing antibodies is cumbersome and time-consuming. Currently, corresponding tests are only conducted in public health laboratories of some countries and regions.
4. **Antigen detection**: After the infected tissue is fixed and embedded, the adenovirus nuclear inclusion body and related antigens can be tested by immunohistochemistry or fluorescence staining.

4.3.3 Parvovirus

The reported detection rates of parvovirus in endocardial biopsy samples of patients with myocarditis is 11–56% [67]. A serum antibody test is the most commonly used method to diagnose PVB19 infection, and a nucleic acid PCR test can further quantify the virus titer. Viral antigen testing is not widely available currently, and PVB19 virus culture is only conducted in research laboratories. Previous and present infections can be distinguished via the detection of IgM and IgG antibodies [69]. At present, HBoV nucleic acid is mainly detected by PCR.

1. Nucleic acid test: One week after PVB19 infection, viral DNA can be detected in the respiratory tract and blood samples of patients. High-titer viremia can last for approximately 1 week, which is then maintained at a low titer level. PCR can be used to diagnose PVB19 infection in the very early stage (before antibody emergence) and is of great value for the diagnosis of PVB19 infection in pregnant women and fetuses. However, low-titer viremia persists after PVB19 infection; thus, viral DNA positivity is not necessarily indicative of present infection. PVB19 virus DNA may also persist in immunocompromised patients. Currently, more than 10^4 viral genomic copies

(vgc)/mL is considered the diagnostic criterion for PVB19 infection. Moreover, the genome of PVB19 has genetic variability, which may affect PCR results.

The first method used to detect PVB19 nucleic acid was dot hybridization, which was gradually replaced by PCR, which is more sensitive. Currently, there are commercially available PCR kits for the diagnosis of PVB19 infection, and the World Health Organization has also established technical standards for nucleic acid amplification of PVB19. In addition to PCR, in situ hybridization can also be used to detect PVB19 DNA in cells or tissues, with a detection sensitivity of about 10^5 vgc/mL.

2. Virus isolation and culture: PVB19 isolation requires special media, such as bone marrow erythroid progenitor cells or embryonic liver cells. Although virus culture is helpful to clarify the infectivity of viruses and study the specific mechanism of virus replication, currently, virus culture is only conducted in laboratory studies due to its finite benefit in clinical diagnosis and treatment and high technical requirements.
3. Serological tests: IgM and IgG antibody tests can be used to diagnose PVB19 infection. However, due to the formation of the antibody–virus complex, serological tests in patients with high-titer viremia may produce false-negative results. Generally, IgM antibodies are produced 7–10 days after PVB19 infection, and IgG antibodies are produced a few days later; positive antibodies can last for 2–4 months. Even if the virus has a genetic mutation, the body's immune response remains; therefore, serological results are unaffected. However, immunocompromised patients may not produce antibodies after infection or may continue to express IgM antibodies without producing IgG antibodies. Even a positive IgG antibody does not rule out passive immunity due to transfusion or intravenous gamma globulin in the active phase of infection.
4. Antigen detection: The detection of virus antigen by immunohistochemistry/fluorescence staining or observation of virus particles through an electron microscope can locate the host cells of the virus, but the sensitivity is relatively low.
5. Bone marrow cytology: Due to the erythrophilic characteristics of PVB19, bone marrow cytology is of great clinical significance. The typical cytological changes of PVB19 infection in bone marrow are erythroid dysplasia resulting from the damage of erythroid precursor cells and emergence of giant erythroid protoblasts containing large eosinophilic nuclear inclusion bodies and cytoplasmic vacuolation.

4.3.4 Human Herpes Virus

The reported detection rate of parvovirus in endocardial biopsy samples of patients with myocarditis is 8–20% [67]. The types of samples currently used to detect HHV-6 infection include the serum/plasma, the cerebrospinal fluid, the alveolar lavage fluid, and various biopsy tissues. The detection methods include the nucleic acid test, virus culture, and serological test. However, due to the prevalence of HHV-6 infection, there is a lack of methods that can be used to identify the incubation period and active period of HHV-6 infection [70].

1. **Nucleic acid detection**: HHV-6 nucleic acid can be detected by PCR or nucleic acid blot hybridization. Southern blot hybridization can be used for the rapid screening of large numbers of samples, but it is not as sensitive as PCR. Currently, PCR primers for different HHV-6 variants are available, which can sensitively identify different virus strains. Due to the high sensitivity of PCR, it is also easy to obtain false-positive results if samples are improperly stored or contaminated.
2. **Virus isolation and culture**: The lymphocytes of patients infected with HHV-6 can be isolated and cultured to obtain virus strains, but this method is time consuming and expensive, has low sensitivity, and is not used for routine diagnosis.

3. **Serological testing**: Currently, there are technical standards for HHV-6 antibody detection, including the anti-complement immunofluorescence method, competitive radioimmunoassay, and the neutralizing antibody method. The disadvantage of serological antibody testing is that HHV-6A cannot be distinguished from HHV-6B infection and may cross-react with HHV-7 to produce false-positive results. In addition, due to the widespread HHV-6 infection, almost all individuals aged over 2 years are positive for HHV-6 IgG antibodies. Typically, a positive IgM antibody indicates a new infection within 5–7 days, but some people do not produce IgM antibodies even if they are infected, which makes it difficult to interpret serological test results.
4. **Antigen detection**: The detection of HHV virus antigens in biopsy tissue samples contributes to observing the changes of tissues at different time points and even the entire infection period. In situ immunohistochemistry/fluorescence tests of infected tissues are often conducted in research laboratories to determine the pathological course of HHV infection rather than as routine clinical diagnostic tests.

Cytokine detection: Cytokine storm plays a significant role in the pathogenesis of FM [71]. The circulating cytokine levels of patients with FM are the primary index that reflects the severity of systemic inflammatory response. The measurement of circulating cytokines would help judge the disease course and assist in the creation of a better treatment plan.

4.3.4.1 Enzyme-Linked Immunosorbent Assay

Principle The assay uses a solid-phase type of enzyme immunoassay to detect the presence of a ligand in a liquid sample using antibodies directed against the protein to be measured. This enzyme-labeled antigen or antibody retains both immune and enzyme activity. Due to the high frequency of enzyme catalysis, it can amplify the reaction effect so that the determination method can reach a high sensitivity.

Advantages Avoids direct labeling with specific antibodies and is cheap.

Disadvantages A large amount of sample is required, only one cytokine can be measured at a time, operation steps and measurement times are very long, and artificial artifacts can be caused by the enzyme-linked reaction.

4.3.4.2 Flow Cytometry

Principle Uses tiny, dispersed particles to capture the liquid analyte and a flow cytometer to detect the fluorescence emitted by the "sandwich" particle–analyte complex to determine the quantity of the analyte.

Advantages Small sample volume required, fast, simple operation, high sensitivity, good repeatability, high efficiency, safe, and close to biological analysis conditions.

Disadvantages Relatively expensive.

4.3.4.3 Liquid Cytokine Chip

Principle This detection method is the combination of enzyme-linked immunosorbent assay and biochip technology based on microplates. It links highly specific capture monoclonal antibodies to different fluorescently labeled magnetic beads and then mixes magnetic beads and suspends them in a microwell plate. Further, a biotin-labeled high-affinity paired detection antibody is added to combine with SA-PE and amplify the signal to achieve the detection of a variety of cytokines simultaneously.

Advantages Small sample volume required, highly flexible panel design, good repeatability, and high efficiency.

Disadvantages Relatively expensive.

4.4 COVID-19 Pandemic-Related Etiology

COVID-19 is caused by SARS-CoV-2, which attacks the respiratory system and other important organs, including the heart [72]. Previous epidemiological data have shown that 7–28% patients have evidence of cardiac injury [73–75]. Although several cases have reported that SARS-CoV-2 could induce myocarditis, relative clinical data is scarce [76–78].

A previous study containing 39 consecutive autopsy cases from Germany indicated that SARS-CoV-2 could be detected in 24 of 39 hearts, with 16 of 39 (41%) having copy numbers higher than 1000 copies/ug RNA [79]. However, there is no direct evidence that SARS-CoV-2 can directly invade cardiomyocytes. Thus, myocardial injury may induce the immune system via hyperactivation characterized by the release of multiple inflammatory mediators [80]. The detailed mechanisms underlying the etiology and pathogenesis of SARS-CoV-2-induced FM requires further exploration.

References

1. Karjalainen J, Heikkila J. Incidence of three presentations of acute myocarditis in young men in military service—a 20-year experience. Eur Heart J. 1999;20:1120–5. https://doi.org/10.1053/euhj.1998.1444.
2. Crowell RL, Landau BJ. A short history and introductory background on the coxsackieviruses of group B. Curr Top Microbiol Immunol. 1997;223:1–11. https://doi.org/10.1007/978-3-642-60687-8_1.
3. Schultz JC, Hilliard AA, Cooper LT, Rihal CS. Diagnosis and treatment of viral myocarditis. Mayo Clin Proc. 2009;84:1001–9.
4. Andreoletti L, Venteo L, Douche-Aourik F, Canas F, Lorin de la Grandmaison G, Jacques J, Moret H, Jovenin N, Mosnier JF, Matta M, et al. Active Coxsackieviral B infection is associated with disruption of dystrophin in endomyocardial tissue of patients who died suddenly of acute myocardial infarction. J Am Coll Cardiol. 2007;50:2207–14. https://doi.org/10.1016/j.jacc.2007.07.080.
5. Kuhl U, Pauschinger M, Noutsias M, Seeberg B, Bock T, Lassner D, Poller W, Kandolf R, Schultheiss HP. High prevalence of viral genomes and multiple viral infections in the myocardium of adults with "idiopathic" left ventricular dysfunction. Circulation. 2005;111:887–93. https://doi.org/10.1161/01.Cir.0000155616.07901.35.
6. Bowles NE, Ni J, Kearney DL, Pauschinger M, Schultheiss H-P, McCarthy R, Hare J, Bricker JT, Bowles KR, Towbin JA. Detection of viruses in myocardial tissues by polymerase chain reaction. Evidence of adenovirus as a common cause of myocarditis in children and adults. J Am Coll Cardiol. 2003;42:466–72. https://doi.org/10.1016/s0735-1097(03)00648-x.
7. Breinholt JP, Moulik M, Dreyer WJ, Denfield SW, Kim JJ, Jefferies JL, Rossano JW, Gates CM, Clunie SK, Bowles KR, et al. Viral epidemiologic shift in inflammatory heart disease: the increasing involvement of parvovirus B19 in the myocardium of pediatric cardiac transplant patients. J Heart Lung Transplant. 2010;29:739–46. https://doi.org/10.1016/j.healun.2010.03.003.
8. Matsumori A, Shimada T, Chapman NM, Tracy SM, Mason JW. Myocarditis and heart failure associated with hepatitis C virus infection. J Card Fail. 2006;12:293–8. https://doi.org/10.1016/j.cardfail.2005.11.004.
9. Bratincsak A, El-Said HG, Bradley JS, Shayan K, Grossfeld PD, Cannavino CR. Fulminant myocarditis associated with pandemic H1N1 influenza a virus in children. J Am Coll Cardiol. 2010;55:928–9. https://doi.org/10.1016/j.jacc.2010.01.004.
10. Veronese G, Ammirati E, Brambatti M, Merlo M, Cipriani M, Potena L, Sormani P, Aoki T, Sugimura K, Sawamura A, et al. Viral genome search in myocardium of patients with fulminant myocarditis. Eur J Heart Fail. 2020; https://doi.org/10.1002/ejhf.1738.
11. Heidecker B, Williams SH, Jain K, Oleynik A, Patriki D, Kottwitz J, Berg J, Garcia JA, Baltensperger N, Lovrinovic M, et al. Virome sequencing in patients with myocarditis. Circ Heart Fail. 2020;13:e007103. https://doi.org/10.1161/CIRCHEARTFAILURE.120.007103.
12. Chen C, Zhou Y, Wang DW. SARS-CoV-2: a potential novel etiology of fulminant myocarditis. Herz. 2020;45:230–2. https://doi.org/10.1007/s00059-020-04909-z.
13. Nikonov OS, Chernykh ES, Garber MB, Nikonova EY. Enteroviruses: classification, diseases they cause, and approaches to development of antiviral drugs. Biochemistry (Mosc). 2017;82:1615–31. https://doi.org/10.1134/S0006297917130041.
14. Pelletier J, Sonenberg N. Internal initiation of translation of eukaryotic mRNA directed by a sequence derived from poliovirus RNA. Nature. 1988;334:320–5. https://doi.org/10.1038/334320a0.
15. Noor A, Krilov LR. Enterovirus infections. Pediatr Rev. 2016;37:505–15. https://doi.org/10.1542/pir.2016-0103.

16. Lynch JP 3rd, Kajon AE. Adenovirus: epidemiology, global spread of novel serotypes, and advances in treatment and prevention. Semin Respir Crit Care Med. 2016;37:586–602. https://doi.org/10.1055/s-0036-1584923.
17. Henquell C, Boeuf B, Mirand A, Bacher C, Traore O, Dechelotte P, Labbe A, Bailly JL, Peigue-Lafeuille H. Fatal adenovirus infection in a neonate and transmission to health-care workers. J Clin Virol. 2009;45:345–8. https://doi.org/10.1016/j.jcv.2009.04.019.
18. Kajon AE, Hang J, Hawksworth A, Metzgar D, Hage E, Hansen CJ, Kuschner RA, Blair P, Russell KL, Jarman RG. Molecular epidemiology of adenovirus type 21 respiratory strains isolated from us military trainees (1996-2014). J Infect Dis. 2015;212:871–80. https://doi.org/10.1093/infdis/jiv141.
19. Verdonschot J, Hazebroek M, Merken J, Debing Y, Dennert R, Brunner-La Rocca HP, Heymans S. Relevance of cardiac parvovirus B19 in myocarditis and dilated cardiomyopathy: review of the literature. Eur J Heart Fail. 2016;18:1430–41. https://doi.org/10.1002/ejhf.665.
20. Ackermann M, Wagner WL, Rellecke P, Akhyari P, Boeken U, Reinecke P. Parvovirus B19-induced angiogenesis in fulminant myocarditis. Eur Heart J. 2020; https://doi.org/10.1093/eurheartj/ehaa092.
21. Young NS, Brown KE. Parvovirus B19. N Engl J Med. 2004;350:586–97. https://doi.org/10.1056/NEJMra030840.
22. Kaufmann B, Simpson AA, Rossmann MG. The structure of human parvovirus B19. Proc Natl Acad Sci U S A. 2004;101:11628–33. https://doi.org/10.1073/pnas.0402992101.
23. Cohen BJ, Buckley MM. The prevalence of antibody to human parvovirus B19 in England and Wales. J Med Microbiol. 1988;25:151–3. https://doi.org/10.1099/00222615-25-2-151.
24. Rohrer C, Gartner B, Sauerbrei A, Bohm S, Hottentrager B, Raab U, Thierfelder W, Wutzler P, Modrow S. Seroprevalence of parvovirus B19 in the German population. Epidemiol Infect. 2008;136:1564–75. https://doi.org/10.1017/S0950268807009958.
25. Mossong J, Hens N, Friederichs V, Davidkin I, Broman M, Litwinska B, Siennicka J, Trzcinska A, Vand P, Beutels P, et al. Parvovirus B19 infection in five European countries: seroepidemiology, force of infection and maternal risk of infection. Epidemiol Infect. 2008;136:1059–68. https://doi.org/10.1017/S0950268807009661.
26. Braun DK, Dominguez G, Pellett PE. Human herpesvirus 6. Clin Microbiol Rev. 1997;10:521–67. https://doi.org/10.1128/CMR.10.3.521.
27. Zerr DM, Meier AS, Selke SS, Frenkel LM, Huang ML, Wald A, Rhoads MP, Nguy L, Bornemann R, Morrow RA, et al. A population-based study of primary human herpesvirus 6 infection. N Engl J Med. 2005;352:768–76. https://doi.org/10.1056/NEJMoa042207.
28. Levy JA, Ferro F, Greenspan D, Lennette ET. Frequent isolation of HHV-6 from saliva and high seroprevalence of the virus in the population. Lancet. 1990;335:1047–50. https://doi.org/10.1016/0140-6736(90)92628-u.
29. Briggs M, Fox J, Tedder RS. Age prevalence of antibody to human herpesvirus 6. Lancet. 1988;1:1058–9. https://doi.org/10.1016/s0140-6736(88)91883-1.
30. Hall CB, Long CE, Schnabel KC, Caserta MT, McIntyre KM, Costanzo MA, Knott A, Dewhurst S, Insel RA, Epstein LG. Human herpesvirus-6 infection in children. A prospective study of complications and reactivation. N Engl J Med. 1994;331:432–8. https://doi.org/10.1056/NEJM199408183310703.
31. Kociol RD, Cooper LT, Fang JC, Moslehi JJ, Pang PS, Sabe MA, Shah RV, Sims DB, Thiene G, Vardeny O, et al. Recognition and initial management of fulminant myocarditis: a scientific statement from the American Heart Association. Circulation. 2020;141:e69–92. https://doi.org/10.1161/CIR.0000000000000745.
32. Moslehi JJ. Cardiovascular toxic effects of targeted cancer therapies. N Engl J Med. 2016;375:1457–67. https://doi.org/10.1056/NEJMra1100265.
33. Bristow MR, Thompson PD, Martin RP, Mason JW, Billingham ME, Harrison DC. Early anthracycline cardiotoxicity. Am J Med. 1978;65:823–32. https://doi.org/10.1016/0002-9343(78)90802-1.
34. Wang DY, Okoye GD, Neilan TG, Johnson DB, Moslehi JJ. Cardiovascular toxicities associated with cancer immunotherapies. Curr Cardiol Rep. 2017;19:21. https://doi.org/10.1007/s11886-017-0835-0.
35. Vanpouille-Box C, Lhuillier C, Bezu L, Aranda F, Yamazaki T, Kepp O, Fucikova J, Spisek R, Demaria S, Formenti SC, et al. Trial watch: immune checkpoint blockers for cancer therapy. Onco Targets Ther. 2017;6:e1373237. https://doi.org/10.1080/2162402X.2017.1373237.
36. Postow MA, Longo DL, Sidlow R, Hellmann MD. Immune-related adverse events associated with immune checkpoint blockade. N Engl J Med. 2018;378:158–68. https://doi.org/10.1056/NEJMra1703481.
37. Johnson DB, Balko JM, Compton ML, Chalkias S, Gorham J, Xu Y, Hicks M, Puzanov I, Alexander MR, Bloomer TL, et al. Fulminant myocarditis with combination immune checkpoint blockade. N Engl J Med. 2016;375:1749–55. https://doi.org/10.1056/NEJMoa1609214.
38. Escudier M, Cautela J, Malissen N, Ancedy Y, Orabona M, Pinto J, Monestier S, Grob JJ, Scemama U, Jacquier A, et al. Clinical features, management, and outcomes of immune checkpoint inhibitor-related cardiotoxicity. Circulation. 2017;136:2085–7. https://doi.org/10.1161/circulationaha.117.030571.
39. Mahmood SS, Fradley MG, Cohen JV, Nohria A, Reynolds KL, Heinzerling LM, Sullivan RJ, Damrongwatanasuk R, Chen CL, Gupta D, et al. Myocarditis in patients treated with immune checkpoint inhibitors. J Am Coll Cardiol. 2018;71:1755–64. https://doi.org/10.1016/j.jacc.2018.02.037.

40. Ball S, Ghosh RK, Wongsaengsak S, Bandyopadhyay D, Ghosh GC, Aronow WS, Fonarow GC, Lenihan DJ, Bhatt DL. Cardiovascular toxicities of immune checkpoint inhibitors: jacc review topic of the week. J Am Coll Cardiol. 2019;74:1714–27. https://doi.org/10.1016/j.jacc.2019.07.079.
41. Simons KH, de Jong A, Jukema JW, de Vries MR, Arens R, Quax PHA. T cell co-stimulation and co-inhibition in cardiovascular disease: a double-edged sword. Nat Rev Cardiol. 2019;16:325–43. https://doi.org/10.1038/s41569-019-0164-7.
42. Moslehi JJ, Salem JE, Sosman JA, Lebrun-Vignes B, Johnson DB. Increased reporting of fatal immune checkpoint inhibitorassociated myocarditis. Lancet. 2018;391:933. https://doi.org/10.1016/S0140-6736(18)30533-6.
43. Koelzer VH, Rothschild SI, Zihler D, Wicki A, Willi B, Willi N, Voegeli M, Cathomas G, Zippelius A, Mertz KD. Systemic inflammation in a melanoma patient treated with immune checkpoint inhibitors-an autopsy study. J Immunother Cancer. 2016;4:13. https://doi.org/10.1186/s40425-016-0117-1.
44. Mehta A, Gupta A, Hannallah F, Koshy T, Reimold S. Myocarditis as an immune-related adverse event with ipilimumab/nivolumab combination therapy for metastatic melanoma. Melanoma Res. 2016;26:319–20. https://doi.org/10.1097/CMR.0000000000000251.
45. Heinzerling L, Ott PA, Hodi FS, Husain AN, Tajmir-Riahi A, Tawbi H, Pauschinger M, Gajewski TF, Lipson EJ, Luke JJ. Cardiotoxicity associated with CTLA4 and PD1 blocking immunotherapy. J Immunother Cancer. 2016;4:50. https://doi.org/10.1186/s40425-016-0152-y.
46. Zimmer L, Goldinger SM, Hofmann L, Loquai C, Ugurel S, Thomas I, Schmidgen MI, Gutzmer R, Utikal JS, Goppner D, et al. Neurological, respiratory, musculoskeletal, cardiac and ocular side-effects of anti-PD-1 therapy. Eur J Cancer. 2016;60:210–25. https://doi.org/10.1016/j.ejca.2016.02.024.
47. Tadokoro T, Keshino E, Makiyama A, Sasaguri T, Ohshima K, Katano H, Mohri M. Acute lymphocytic myocarditis with anti-pd-1 antibody nivolumab. Circ Heart Fail. 2016;9 https://doi.org/10.1161/CIRCHEARTFAILURE.116.003514.
48. Semper H, Muehlberg F, Schulz-Menger J, Allewelt M, Grohe C. Drug-induced myocarditis after nivolumab treatment in a patient with PDL1- negative squamous cell carcinoma of the lung. Lung Cancer. 2016;99:117–9. https://doi.org/10.1016/j.lungcan.2016.06.025.
49. Gibson R, Delaune J, Szady A, Markham M. Suspected autoimmune myocarditis and cardiac conduction abnormalities with nivolumab therapy for non-small cell lung cancer. BMJ Case Rep. 2016;2016 https://doi.org/10.1136/bcr-2016-216228.
50. Schulert GS, Grom AA. Pathogenesis of macrophage activation syndrome and potential for cytokine- directed therapies. Annu Rev Med. 2015;66:145–59. https://doi.org/10.1146/annurev-med-061813-012806.
51. Mehta P, Porter JC, Manson JJ, Isaacs JD, Openshaw PJM, McInnes IB, Summers C, Chambers RC. Therapeutic blockade of granulocyte macrophage colony-stimulating factor in COVID-19-associated hyperinflammation: challenges and opportunities. Lancet Respir Med. 2020; https://doi.org/10.1016/s2213-2600(20)30267-8.
52. Clark IA. The advent of the cytokine storm. Immunol Cell Biol. 2007;85:271–3. https://doi.org/10.1038/sj.icb.7100062.
53. Abe S, Okura Y, Hoyano M, Kazama R, Watanabe S, Ozawa T, Saigawa T, Hayashi M, Yoshida T, Tachikawa H, et al. Plasma concentrations of cytokines and neurohumoral factors in a case of fulminant myocarditis successfully treated with intravenous immunoglobulin and percutaneous cardiopulmonary support. Circ J. 2004;68:1223–6. https://doi.org/10.1253/circj.68.1223.
54. Noji Y. Anakinra in fulminant myocarditis: targeting Interleukin-1 and the inflammasome formation. Crit Care Med. 2016;44:1630–1. https://doi.org/10.1097/CCM.0000000000001769.
55. Hay KA, Hanafi LA, Li D, Gust J, Liles WC, Wurfel MM, Lopez JA, Chen J, Chung D, Harju-Baker S, et al. Kinetics and biomarkers of severe cytokine release syndrome after CD19 chimeric antigen receptor-modified T-cell therapy. Blood. 2017;130:2295–306. https://doi.org/10.1182/blood-2017-06-793141.
56. Hay KA. Cytokine release syndrome and neurotoxicity after CD19 chimeric antigen receptor-modified (CAR-) T cell therapy. Br J Haematol. 2018;183:364–74. https://doi.org/10.1111/bjh.15644.
57. Kindler E, Thiel V, Weber F. Interaction of SARS and MERS coronaviruses with the antiviral interferon response. Adv Virus Res. 2016;96:219–43. https://doi.org/10.1016/bs.aivir.2016.08.006.
58. Channappanavar R, Fehr AR, Vijay R, Mack M, Zhao J, Meyerholz DK, Perlman S. Dysregulated type I interferon and inflammatory monocyte-macrophage responses cause lethal pneumonia in SARS-CoV-infected mice. Cell Host Microbe. 2016;19:181–93. https://doi.org/10.1016/j.chom.2016.01.007.
59. Channappanavar R, Perlman S. Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology. Semin Immunopathol. 2017;39:529–39. https://doi.org/10.1007/s00281-017-0629-x.
60. Bermejo-Martin JF, Ortiz de Lejarazu R, Pumarola T, Rello J, Almansa R, Ramirez P, Martin-Loeches I, Varillas D, Gallegos MC, Seron C, et al. Th1 and Th17 hypercytokinemia as early host response signature in severe pandemic influenza. Crit Care. 2009;13:R201. https://doi.org/10.1186/cc8208.
61. Grom AA, Horne A, De Benedetti F. Macrophage activation syndrome in the era of biologic therapy. Nat Rev Rheumatol. 2016;12:259–68. https://doi.org/10.1038/nrrheum.2015.179.
62. Esfandiarei M, McManus BM. Molecular biology and pathogenesis of viral myocarditis. Annu Rev Pathol.

2008;3:127–55. https://doi.org/10.1146/annurev.pathmechdis.3.121806.151534.
63. Cooper DJ. Myocarditis. N Engl J Med. 2009;
64. Fung G, Luo H, Qiu Y, Yang D, McManus B. Myocarditis. Circ Res. 2016;118:496–514. https://doi.org/10.1161/circresaha.115.306573.
65. Koonin EV, Wolf YI, Nagasaki K, Dolja VV. The big bang of picorna-like virus evolution antedates the radiation of eukaryotic supergroups. Nat Rev Microbiol. 2008;6:925–39. https://doi.org/10.1038/nrmicro2030.
66. Bowles NE, Rose ML, Taylor P, Banner NR, Morgan-Capner P, Cunningham L, Archard LC, Yacoub MH. End-stage dilated cardiomyopathy. Persistence of enterovirus RNA in myocardium at cardiac transplantation and lack of immune response. Circulation. 1989;80:1128–36. https://doi.org/10.1161/01.Cir.80.5.1128.
67. Pankuweit S, Klingel K. Viral myocarditis: from experimental models to molecular diagnosis in patients. Heart Fail Rev. 2012;18:683–702. https://doi.org/10.1007/s10741-012-9357-4.
68. Lee J, Choi EH, Lee HJ. Comprehensive serotyping and epidemiology of human adenovirus isolated from the respiratory tract of Korean children over 17 consecutive years (1991-2007). J Med Virol. 2010;82:624–31. https://doi.org/10.1002/jmv.21701.
69. Gallinella G, Zuffi E, Gentilomi G, Manaresi E, Venturoli S, Bonvicini F, Cricca M, Zerbini M, Musiani M. Relevance of B19 markers in serum samples for a diagnosis of parvovirus B19-correlated diseases. J Med Virol. 2003;71:135–9. https://doi.org/10.1002/jmv.10452.
70. Ablashi D, Agut H, Alvarez-Lafuente R, Clark DA, Dewhurst S, DiLuca D, Flamand L, Frenkel N, Gallo R, Gompels UA, et al. Classification of HHV-6A and HHV-6B as distinct viruses. Arch Virol. 2014;159:863–70. https://doi.org/10.1007/s00705-013-1902-5.
71. Hang W, Chen C, Seubert JM, Wang DW. Fulminant myocarditis: a comprehensive review from etiology to treatments and outcomes. Signal Transduct Target Ther. 2020;5:287. https://doi.org/10.1038/s41392-020-00360-y.
72. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395:1054–62. https://doi.org/10.1016/s0140-6736(20)30566-3.
73. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H, Cheng Z, Xiong Y, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA. 2020;323:1061–9. https://doi.org/10.1001/jama.2020.1585.
74. Guo T, Fan Y, Chen M, Wu X, Zhang L, He T, Wang H, Wan J, Wang X, Lu Z. Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). JAMA Cardiol. 2020;5:811–8. https://doi.org/10.1001/jamacardio.2020.1017.
75. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395:497–506. https://doi.org/10.1016/S0140-6736(20)30183-5.
76. Zeng JH, Liu YX, Yuan J, Wang FX, Wu WB, Li JX, Wang LF, Gao H, Wang Y, Dong CF, et al. First case of COVID-19 complicated with fulminant myocarditis: a case report and insights. Infection. 2020; https://doi.org/10.1007/s15010-020-01424-5.
77. Garot J, Amour J, Pezel T, Dermoch F, Messadaa K, Felten ML, Raymond V, Baubillier E, Sanguineti F, Garot P. SARS-CoV-2 fulminant myocarditis. JACC Case Rep. 2020;2:1342–6. https://doi.org/10.1016/j.jaccas.2020.05.060.
78. Kesici S, Aykan HH, Orhan D, Bayrakci B. Fulminant COVID-19-related myocarditis in an infant. Eur Heart J. 2020;41:3021. https://doi.org/10.1093/eurheartj/ehaa515.
79. Lindner D, Fitzek A, Brauninger H, Aleshcheva G, Edler C, Meissner K, Scherschel K, Kirchhof P, Escher F, Schultheiss HP, et al. Association of cardiac infection with SARS-CoV-2 in confirmed COVID-19 autopsy cases. JAMA Cardiol. 2020;5:1281–5. https://doi.org/10.1001/jamacardio.2020.3551.
80. Kawakami R, Sakamoto A, Kawai K, Gianatti A, Pellegrini D, Nasr A, Kutys B, Guo L, Cornelissen A, Mori M, et al. Pathological evidence for SARS-CoV-2 as a cause of myocarditis: JACC review topic of the week. J Am Coll Cardiol. 2021;77:314–25. https://doi.org/10.1016/j.jacc.2020.11.031.