Myocarditis and inflammatory cardiomyopathy: from diagnosis to treatment

Miyokardit ve enflamatuvar kardiyomiyopati: Tanıdan tedaviye

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Summary– Based on the definition in the European Society of Cardiology statement, myocarditis is an inflammatory disease of the myocardium diagnosed by established histological, immunological, and immunohistochemical criteria, whereas inflammatory cardiomyopathy is myocarditis in association with cardiac dysfunction. Actual incidences of myocarditis and CMi are difficult to determine. Studies addressing the issue of sudden cardiac death in young people report a highly variable autopsy prevalence of myocarditis, ranging from 2-42% of cases. Similarly, biopsy-proven myocarditis has been reported in 9-16% of adult patients with unexplained nonischemic dilated cardiomyopathy (DCM). In up to 30% of cases, biopsy-proven myocarditis can progress to DCM and is associated with a poor prognosis. Prognosis in myocarditis patients also varies according to underlying etiology.

Özet– Avrupa Kardiyoloji Derneği tarafından bildirilen tanıma göre miyokardit, yerleşmiş histolojik, immünolojik ve immünohistokimyasal ölçütlerle tanısı konan enflamatuvar bir miyokardiyum hastalığı, enflamatuvar kardiyomiyopati ise kalp işlev bozukluğu ile ilişkili miyokarditir. Miyokardit ve enflamatuvar kardiyomiyopatinin gerçek insidansını belirlemek zordur. Gençlerde görülen ani kardiyak ölümleri araştıran çalışmalar, %2 ile %42 arasında, son derece değişken bir miyokardit otopsi prevalansı bildirmektedir. Benzer bir şekilde, açıklanamayan iskemik olmayan dilate kardiyomiyopatisi olan yetişkin hastaların %9-16'sında biyopsi ile kanıtlanmış miyokardit bildirilmiştir. Olguların %30'una kadar varan bir kesiminde, biyopsi ile kanıtlanmış miyokardit dilate kardiyomiyopatiye ilerleyebilmekte ve kötü bir prognoz ile ilişkilidir. Miyokardit hastalarında prognoz ayrıca altta yatan etiyolojiye göre de çeşitlilik göstermektedir.

Causes

Infectious agents are the major causes of myocarditis and inflammatory cardiomyopathy (CMi) in diseases of the heart muscle.[1-9] Viral forms are considered the most common cause of acquired CMi.

For decades, coxsackieviruses, and to a lesser extent adenoviruses, have been well established in pediatric and adult myocarditis and chronic heart muscle disease.[10–12] Furthermore, distinct genotypes of erythroviruses, including parvovirus B19, Human herpesvirus 6 (HHV-6A/B), human immunodeficiency virus, cytomegalovirus, herpes simplex virus type 2, and Hepatitis C virus among many others, have been identified with varying degrees of frequency in cardiac tissues.

Clinical presentation and diagnosis

Myocarditis and CMi are challenging diagnoses due to heterogeneity of clinical presentations. Clinical presentations of myocarditis and CMi range broadly from subclinical disease to fulminant heart failure, and chest pain, palpitations, and syncope are not uncommon. Myocarditis can manifest like a myocardial infarction, with sudden-onset angina pectoris, arrhythmias, and/or heart failure developing within days. Most patients with myocarditis initially have such non-specific symptoms that these are often categorized in the context of the preceding infection and not as being of cardiac origin. Cardiac involvement is often considered as the differential diagnosis only when cardiac symp-

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toms, such as palpitations, angina, and/or exertional dyspnea, persist for a long period after the underlying infection has resolved or if they develop de novo in the course of recovery. At this point, electrocardiography results and laboratory chemical findings characteristic of acute myocarditis, such as changes to the ST segment and raised cardiac enzymes typical of acute myocardial involvement, are no longer present.

Abbreviations:

B19V	*Human parvovirus B19*
ciHHV-6	*Chromosomally integrated HHV-6*
CMi	*Cardiomyopathy*
DCM	*Dilated cardiomyopathy*
DNA	*Deoxyribonucleic acid*
EMB	*Endomyocardial biopsy*
HHV-6	*Human herpesvirus 6*
IFN-β	*Interferon beta*
LVEF	*Left ventricular ejection fraction*
miRNA	*MicroRNA*
NGS	*Next-generation sequencing*
PCR	*Polymerase chain reaction*
RNA	*Ribonucleic acid*

Acute, infarct-like changes to electrocardiogram, positive troponin T/I measurement, raised NT-proBNP, and a finding of edema or early contrast enhancement in patients with clinically suspected myocarditis non-specifically indicates virus-associated or inflammatory cell-associated injury to the myocardium. However, information has neither been provided regarding type of infectious pathogen or inflammation, or whether the infectious strain had been completely eliminated or the inflammation subsided. Unequivocally confirmed bioptic diagnosis is the crucial prerequisite for differential diagnostic evaluation and specific treatment strategies derived from this.

In case of occurrence of myocardial damage for which no specific treatment options exist, such as postinfectious or postinflammatory dilated cardiomyopathy (DCM) diagnosed too late, development or progression of heart failure in the long term cannot be prevented.[13]

Endomyocardial biopsy

Endomyocardial biopsy (EMB) remains the gold standard for the diagnosis of myocarditis and CMi. EMB is the only diagnostic tool for establishing etiological diagnosis (viral or immune-mediated) in myocarditis and CMi. Therefore, this implies that all patients with suspected myocarditis should undergo EMB, which is not routine practice. Current guidelines recommend EMB only in a limited number of clinical scenarios that do not include certain common presentations of myocarditis, particularly pseudo-infarction.[4] Actually, the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases recommends that selective coronary angiography and EMB be performed in all patients fulfilling diagnostic criteria for clinically suspected myocarditis.[1] EMB confirms diagnosis of myocarditis, and identifies underlying etiology and type of inflammation, which suggest different treatments and prognosis. An incomplete diagnosis may provide an incomplete picture of the disease, leading to misinterpretation and possibly incorrect treatment decisions.

EMB can be performed with a very low major complication rate when performed by highly experienced operators.[14] In experienced hands, left ventricular biopsy is as safe as right ventricular biopsy.[15]

Basic prerequisite for clinically relevant biopsy diagnostic is the removal of a sufficient number (more than 10 biopsies) of sufficiently large (2–3 mm²), high-quality tissue samples from different areas of the myocardium. If the sample predominantly contains thrombus, fat, or connective tissue, additional samples are required.[16] Apart from specific conditions such as arrhythmogenic right ventricular dysplasia/cardiomyopathy, in which characteristic tissue changes mainly occur in certain myocardial areas of the right ventricle,[17] tissue diagnostic for most questions has similar sensitivity for left or right ventricular biopsy.[18] To what extent targeted biopsy of abnormal diagnostic areas of the myocardium, e.g. regional wall motion abnormality, improves sensitivity of biopsy diagnostic is subject of ongoing investigations.

Highly complex staged diagnostics of endomyocardial biopsies should be performed in specialized and certified laboratories with standardized protocols under constant entrainment of internal control samples in all work areas. Constant participation in interlaboratory surveys for laboratory testing methods and regular inspections of laboratories by independent monitoring bodies ensure quality, and thus significance, of tissue diagnostics (Figure 1).

Fixation of biopsies for histological investigations is directly performed in buffered 4–10% formaldehyde at room temperature. For immunohistological inflammation diagnostics and molecular studies, fixation in RNAlater (Thermo Fisher Scientific Inc., Cambridge, MA, USA) is necessary. RNAlater is particularly suitable for preservation of unstable ribonucleic acid (RNA) at room temperature.

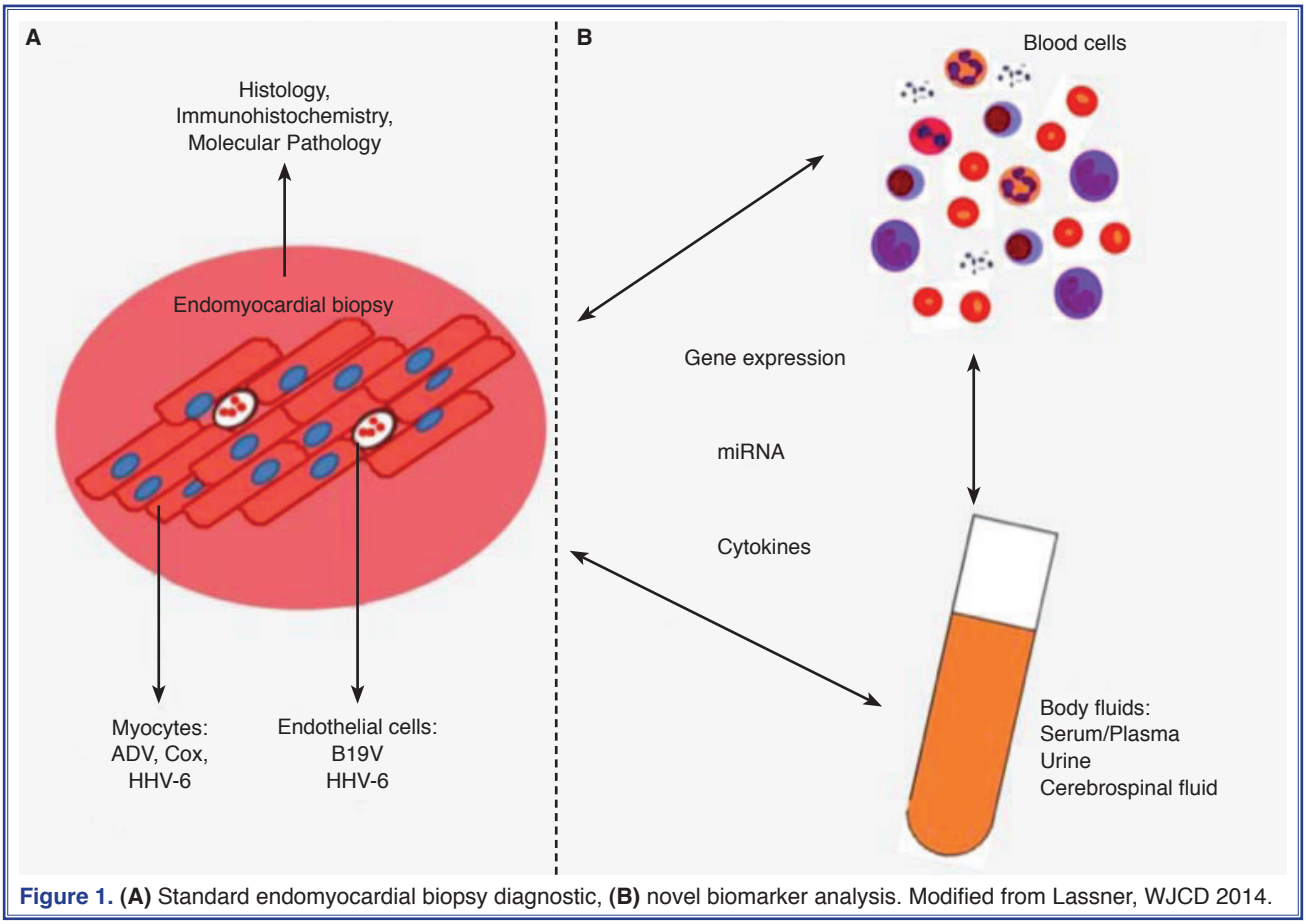

Figure 1. **(A)** Standard endomyocardial biopsy diagnostic, **(B)** novel biomarker analysis. Modified from Lassner, WJCD 2014.

Histological analysis

Histological examination of paraffin sections by different staining protocols (hematoxylin and eosin, elastic van Gieson, periodic acid–Schiff, Azan) detects myocardial cell death, scars, fibrosis, disarrays, cardiomyocytes changes, pathological vascular conditions, granulomas, and inflammatory cell differentiation. Storage disorders, such as amyloidosis, iron deposits, glycogen, and others, can be excluded or specified by additional staining, e.g., immunohistochemical differentiation of amyloid subtypes and optional electron microscopic analyses. EMB diagnosis of myocarditis was based on histomorphological criteria according to Dallas classification.[19] Active myocarditis is defined by simultaneous detection of inflammatory infiltrates and present lysis of cardiomyocytes.

Immunohistochemical examination on intramyocardial inflammation

Whereas Dallas criteria based on light microscopy are limited by interobserver variability in interpreting biopsy specimens,[20] with use of immunohistochemistry, number of EMBs revealing diagnoses of myocarditis and CMi markedly increased. Monoclonal antibodies allow exact characterization and localization of cell infiltrates and cell adhesion molecules, which are relevant for prognosis. Immunohistochemical diagnostics are based on application of specific primary antibodies. Secondary antibody is conjugated with an enzyme complex producing a precipitating colored complex by use of staining solution.

Colored immunospots are counted digitally by application of established digital imaging analysis software for calculating area fractions, numbers of immunospots, and area of myocardial tissue. Digital imaging system consists of microscopic unit, digital camera, and supporting analyzing software.[21,22]

Immunohistochemical analyses are carried out on frozen sections (two EMBs) in order to allow detection of elevated inflammatory cell subsets including non-paraffin staining antibodies, e.g. CD3, CD11a (LFA-1), CD11b (Mac-1), CD45R0 (memory or acti-

vated lymphocytes), perforin-positive cytotoxic lymphocytes, and increased expression of adhesion molecules CD54 (ICAM), CD106 (VCAM), and HLA-1 as marker for tissue activation (Figure 2).

Molecular virology for detection of myocardial infections

Microbial genomes are determined, quantified, and sequenced using methods based on polymerase chain reaction (PCR). Nested PCR protocols consist of 2 sequentially performed PCR assays, where amplicon of first assay is template for second reaction. This procedure is highly sensitive and enables us to detect very low copy numbers of viral genomes.

Depending on the 2 types of viral nucleic acids, isolation of deoxyribonucleic acid (DNA) and RNA is performed in separate extraction procedures. Current viral load monitoring for effective treatment is performed by quantitative PCR. Transcriptional activity of virus in myocardial tissue or peripheral blood cells will be routinely determined for Erythrovirus and HHV-6, the 2 most common cardiotropic viruses, by nested real-time PCR and qPCR. Patients with active erythroviral infection reported a higher frequency of chest pain, systolic left ventricular ejection fraction (LVEF) and accompanied increase of left ventricular end-diastolic diameter. Because these differences were not associated with myocardial inflammation, viral replication seems to be an independent prognostic marker.[23]

HHV-6 A/B are possible pathogenetic causes of myocarditis and idiopathic-cardiomyopathy subsets.

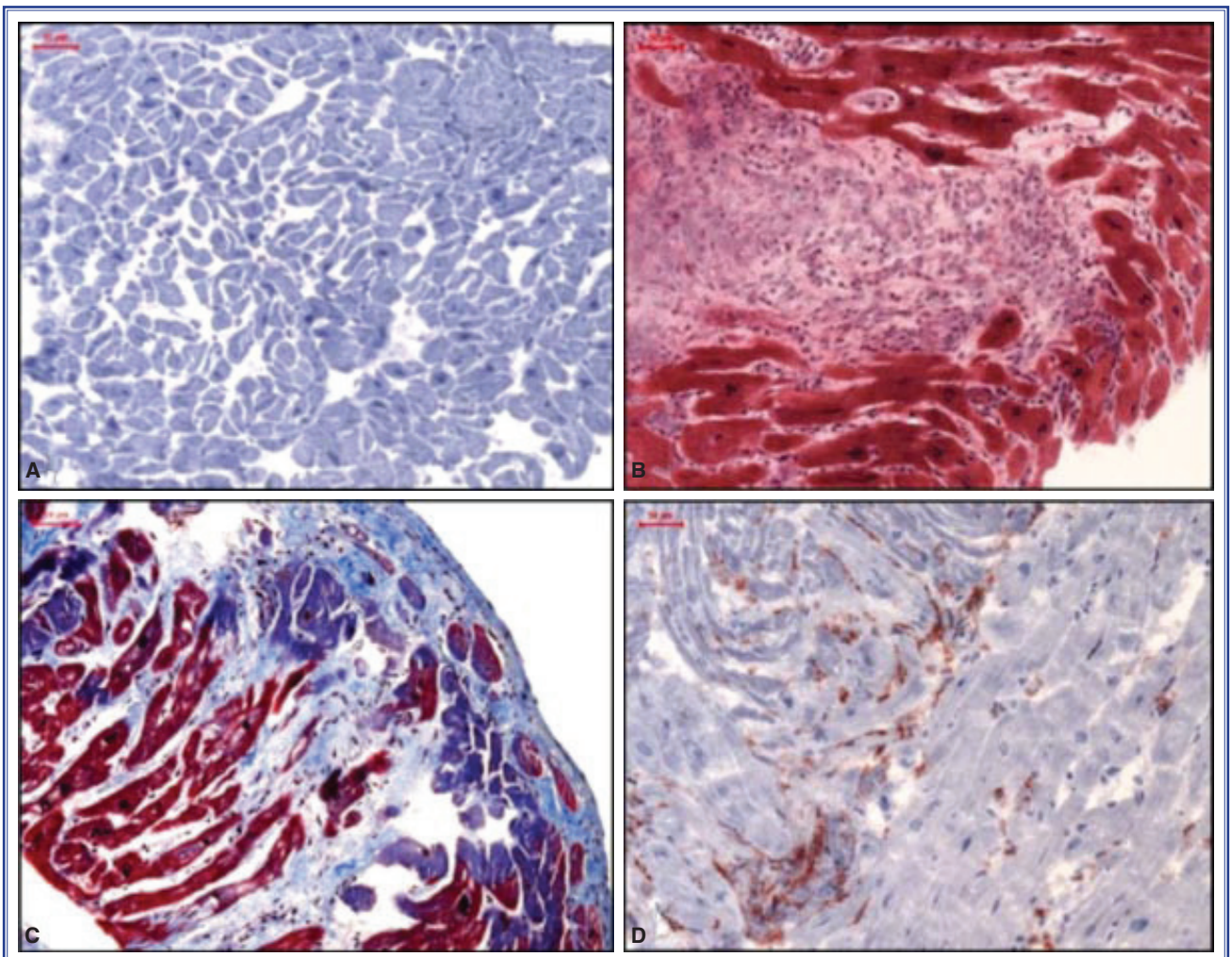

Figure 2. Representative images: **(A)** healed myocarditis, magnification x200, **(B)** active myocarditis, Azan staining, magnification x200, **(C)** dilated cardiomyopathy, Azan staining, magnification x200, **(D)** inflammatory cardiomyopathy, immunohistochemical staining of CD3-positive T-lymphocytes, magnification x200.

[9,24] Prevalence of chromosomally integrated HHV-6 (ciHHV-6) is approximately 0.8% of HHV-6 positive endomyocardial biopsies. Identifying individuals with ciHHV-6 is important because complete HHV-6 genome is present in every cell,[25] and permanent reactivation of virus in all tissues is assumed. Detection of ciHHV-6 is only possible by qPCR. Persistent high viral loads of HHV-6 genomes in blood cells or tissues exclude active infection and confirm ciHHV-6 presence. Elimination of chromosomally integrated virus is impossible, but transcriptional activity of ciHHV-6 is reduced under treatment with antiviral drug. Monitoring of viral RNA load is best indicator for effective therapy or reactivation of ciHHV-6.[26]

Current molecular biological workflow for detection of myocardial infection is combination of qualitative (nPCR) and quantitative detection of viral genomes by nucleic acid isolation systems, thermocycling, gel electrophoresis, and real-time qPCR, and subsequent sequencing of generated PCR amplicons by DNA sequencing devices (Figure 3; Table 1).

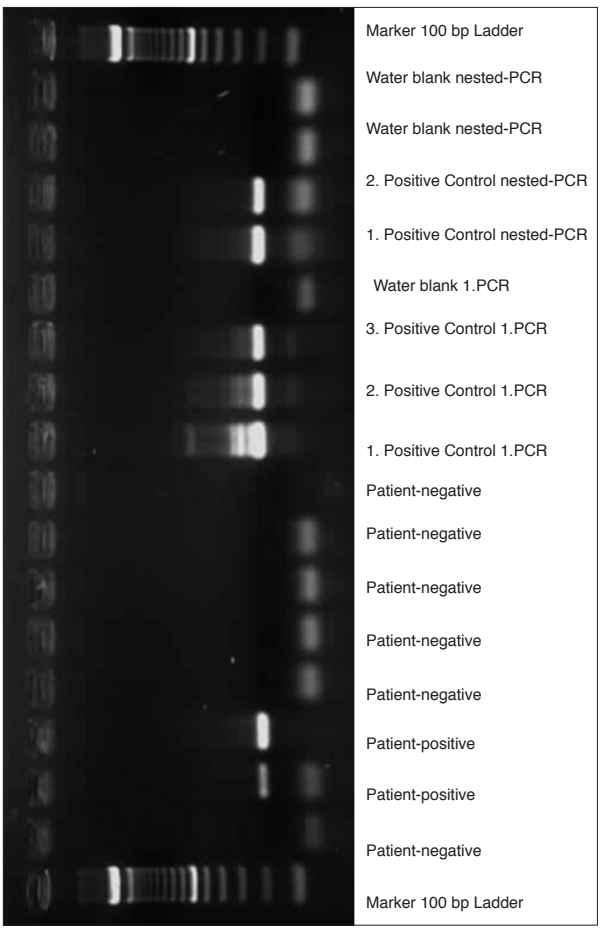

Figure 3. Example of diagnostic nested PCR coxsackievirus.

Pathomechanisms and prognosis

Prognosis of myocarditis and CMi depends on etiology, clinical presentation, and disease stage. While myocarditis and CMi patients can have partial or full clinical recovery, some may relapse many years after the first episode.[27,28]

Molecular biology diagnostic testing for causative agent is done by means of nPCR and identifies rel-

Table 1. Routine biopsy diagnostics

Histology
H&E
Elestica-van-(EvG)
PAS Azan
Congored
v. Kossa
Alcianblue
Stain on iron
SiriusRed
Immunohistochemistry
CD3
LFA1
Mac-1
Perforin
HLA-1
CD54
CD 106
CD31
Collagen1/Collagen 3
Desmosomal proteins for ARVD diagnostics
Molecular Virology/Microbiology
Adenovirus
Coxsackievirus
Erythrovirus (Parvovirus B19) and viral replication
Human Herpesvirus 6 (HHV6)
Chromosomally integrated Human Herpesvirus 6
(ciHHV6)
Epstein-Barr-Virus (EBV)
Borrelia burgendorferi
Hepatitis B and C
CMV
Other

evant infectious pathogens with very high degree of sensitivity. Qualitative diagnosis of viral pathogens is complemented by quantitatively determining viral load (real-time PCR) and sequencing for the purpose of identifying viral subtypes or quality assurance. Acute or latent infections and infections that replicate actively in the myocardium can be differentiated from one another by parallel analyses of blood composition (peripheral cells, plasma, serum) and confirmation of transcriptional activity.

After enteroviral (CVB3) entry, acute injury of myocytes, induced by virus replication, leads to myocyte necrosis and activation of the immune system, which is characterized by invasion of natural killer cells and macrophages, followed by T lymphocytes. Acute phase of myocarditis takes only a few days. After acute phase of virus-induced injury, second phase is characterized by (auto-) immune reactions. This subacute phase, which covers a few weeks to several months, is defined by activated virus-specific T lymphocytes, which may target the host's organs by molecular mimicry.[29]

Persistence of Enterovirus in the myocardium has been associated with ventricular dysfunction and viral genome clearance with improvement of ventricular function and 10-year prognosis. As viruses and viral subtypes respond differently to antiviral medications, and are in some cases not completely eliminated, merely blocked in their continual replication, this information is important for making a tailored decision regarding treatment and success thereof.[30]

Clinical impact of vasculotropic Human parvovirus B19 (B19V) in the heart is still under discussion. However, we demonstrated that in patients with CMi and significantly affected systolic function, presence of B19V-infected endothelial cells was associated with impaired outcome, compared to virus-negative patients presenting with similar frequency and grade of cellular inflammation. Underlying pathogenetic mechanisms are unknown, but may involve either aggravation of inflammatory cell-associated myocardial injury or affect outcome due to additional chronic endothelial dysfunction, both of which can be caused by vasculotropic viral infection. B19V-infected vascular endothelium may thus represent important cofactor that influences clinical course of inflammatory cardiomyopathy.[31–33]

In a previous study, immunohistological evidence of inflammation was identified as an independent predictor of survival.[34,35] Moreover, exact quantification of intramyocardial infiltration is relevant for prognosis. Histology on paraffin section and parallel analysis on cryosection, by set of 6–8 specific antibodies staining inflammatory cells and adhesion molecules for subsequent digital imaging analysis, allow characterization of acute and chronic myocarditis without relevant sampling error. Included are highly prognostic parameters (CD3, perforin, and CD45R0), which predict long-term outcome of patients at time of initial biopsy. Recently, we demonstrated that presence of cytotoxic perforin-positive myocardium-infiltrating cells predicts adverse LVEF course over long follow-up period in a large cohort of 495 CMi patients. This was the first report elucidating prognostic impact of perforin-positive cells for outcome of CMi patients, and it indicated that exact analysis and quantification of intramyocardial infiltrates has clinical value for assessment of long-term LVEF prognosis in CMi.[36] These data should be helpful for clinical practice of cardiologists starting immunosuppressive therapy earlier in high-risk patients (i.e., those with high perforin levels in EMBs).

Besides genetic markers for a limited subgroup of cardiomyopathies, novel biomarkers such as microRNA (miRNA) and gene expression profiling are introduced in molecular examination of EMB, presenting a global picture of the heart muscle and overcoming the limitation of biopsy-focused diagnostics.[23]

It must be emphasized that untreated giant cell and eosinophilic myocarditis have an extremely poor prognosis, with survival rates of less than 20% at 4 years.[37] For diagnosis of giant cell and eosinophilic myocarditis, which are often missed by histological examination, we could develop and routineously apply myocardial gene profiling.[38]

Therefore, such disease-specific profiles will change during effective treatment and could thereby also be applied for therapy monitoring. Recently identified as important regulators of genetic expression in myocardial tissue, miRNAs are small, non-coding regulatory molecules (17–24 nucleotides).[39] They modulate gene expression by enhanced degradation of protein-coding messenger RNAs or sequestration from translational apparatus. Role of miRNAs in physiological and pathological processes and ability to correlate expression changes with disease states,

highlighting their value as novel molecular biomarkers.[40] Currently available miRNA profiles allow identification of preceding cardiotropic infection, even in PCR-negative biopsies. Clinical course of the patient is predictable at point of primary biopsy, and a disease-directed therapy could be initiated immediately to prevent myocardial injuries.

Gene mutations in cardiomyopathies

Current guidelines recommend genetic screening (evidence level A) for arrhythmogenic right ventricular dysplasia/cardiomyopathy, hypertrophic cardiomyopathy, and DCM with conduction abnormalities or extra cardiac manifestations.[41] Next-generation sequencing (NGS) technologies will enable each lab to perform genetic testing for a low price, though interpretation of genetic data still requires great expertise. Now NGS is entering the diagnostic level. Identification of temporary unknown mutations in patients with expressed heart failure problems is the advantage of de novo sequencing of all cardiac genes.

Until now, genetic testing has been generally unable to detect viral infections or inflammation in the myocardium. Nevertheless, the enormous power of NGS to read out millions of sequence fragments in a very short period will allow parallel identification of pathological mutations in genomic DNA and included microbial genomes in the same patient sample.

Treatment options

Mainstay of treatment for CMi is optimal heart failure medical regimen. Moreover, EMB is basis for safe (infection-negative) immunosuppression or antiviral treatment.

Antiviral treatment

Enterovirus and adenoviral infections respond well to interferon beta (IFN-ß).[30] Treatment scheme in chronic viral cardiomyopathy closely follows experiences gained in multiple sclerosis. Initial dose of 2×106 IU IFN-ß is administered subcutaneously every other day and increased at weekly intervals, first to 4×10^6 IU, then to $6–8\times10^6$ IU, continued for 24 weeks. Symptomatic treatment for heart failure is maintained. Slowly increasing dose of IFN-ß, at least initially, or administering non-steroidal anti-

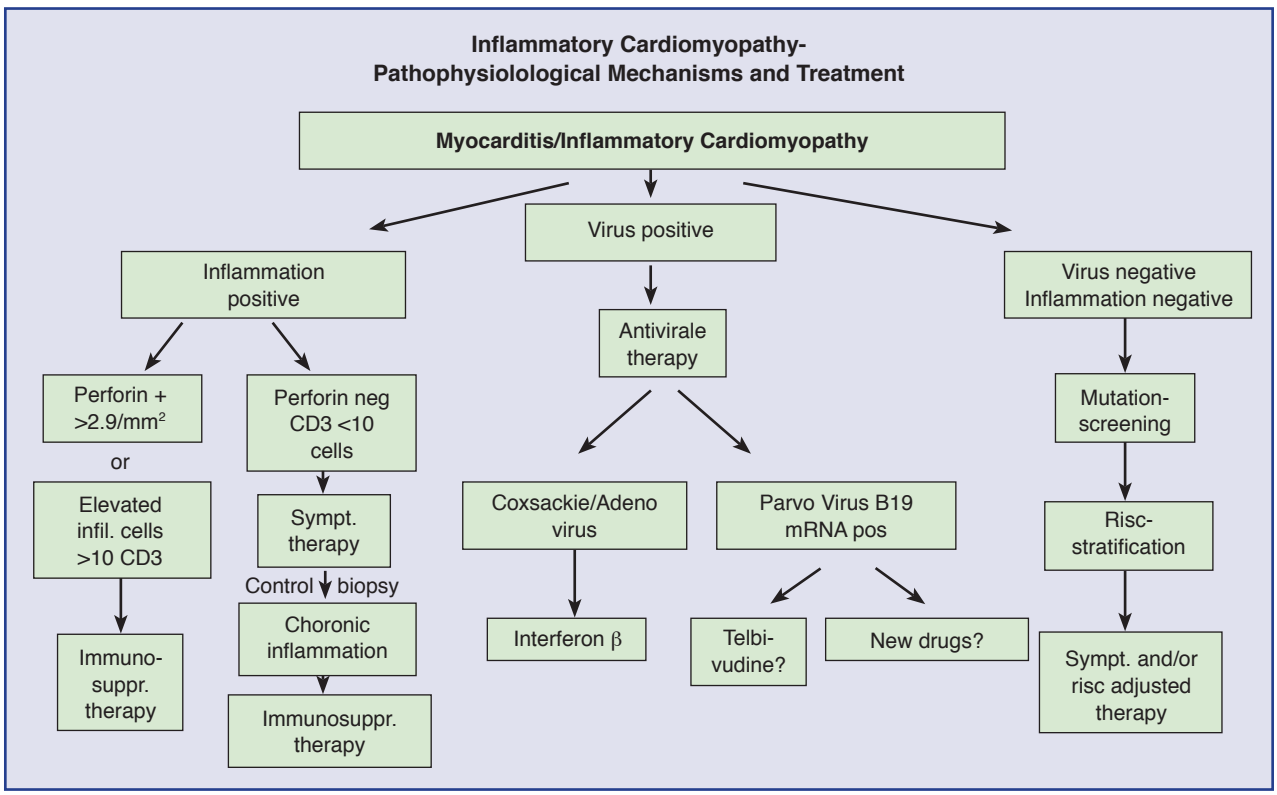

Figure 4. Flow chart of inflammatory cardiomyopathy: Pathophysiological mechanisms and treatment.

rheumatics notably reduces flu-like side effects of medication.

In the first open-label treatment study, 6 months of antiviral treatment of patients positive for Enterovirus or adenovirus showed complete virus elimination and reduction in virus-associated myocardial inflammatory reaction.[41,42] In parallel, significant clinical and hemodynamic improvements were seen in two-thirds of treated patients. Efficacy of antiviral therapy was independent of duration of illness; patients with higher-grade impaired left ventricular pump function (LVEF <45%) especially benefited from treatment.

Immunosuppressive treatment

Currently available data show that immunosuppressive therapy is an effective and safe option for recovery of cardiac failure in carefully selected patients with biopsy-proven, virus-negative inflammatory cardiomyopathy. Administered anti-inflammatory drugs are corticosteroids, azathioprine, and cyclosporine, which are administered on top of regular heart failure medication.

Generally, α-methylprednisolone is administered at a dose of 1 mg/kg body weight, initially for 4 weeks. Depending on body weight, azathioprine is administered at a dose of 100–150 mg daily, in addition to corticosteroid. Steroid dosage is titrated down every 2 weeks in increments of 10 mg until maintenance dose of 10 mg is reached. Treatment duration should last 3–6 months. Actual data of first randomized trials confirm efficacy of treatment regimens in carefully selected patients.[43,44]

A recently published randomized Tailored Immunosuppression in Inflammatory Cardiomyopathy (TIMIC) trial confirmed positive treatment response in patients with chronic active myocarditis. A total of 88% showed improvement of cardiac function and dimensions, defined as increase of >10 percentage points in absolute ejection fraction and reduction of left ventricular end-diastolic volume or left ventricular end-diastolic diameter. At 6 months, no untreated patients showed improvement of LVEF, which significantly worsened compared with baseline.

Moreover, in our EMB-based analysis of CMi patients, immunosuppressive treatment showed effectiveness and beneficial effects, even after long-term follow-up period (up to 10 years). Majority of patients improved significantly with LVEF, especially those with noticeably enhanced inflammation at baseline (data submitted; Figure 4).

Conclusion

Aside from heart failure therapies, in myocarditis and CMi there is no alternative to etiologically driven specific treatment. EMB is the only diagnostic tool for establishing etiological diagnosis (viral or immune-mediated) in myocarditis and CMi. Because clinical course of myocarditis and CMi is unpredictable, all patients with clinically suspected myocarditis and CMi must undergo endomyocardial biopsy before development of irreversible and thus untreatable damage to the myocardium. Exact analysis and quantification of intramyocardial infiltrates, as well as diagnosis of viral pathogens, have high clinical value for assessment of long-term LVEF prognosis and initiation of specific therapy in these diseases.

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