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HUMAN VIRAL CARDIOMYOPATHY

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1. ABSTRACT

Viral infection of the heart is relatively common, usually asymptomatic and has a spontaneous and complete resolution. It can, however, in rare cases, lead to substantial cardiac damage, development of viral cardiomyopathy and

congestive heart failure. Viral cardiomyopathy is defined as viral persistence in a dilated heart. It may be accompanied by myocardial inflammation and then termed inflammatory viral cardiomyopathy (or viral myocarditis with cardiomegaly). If no inflammation is observed in the biopsy of a dilated heart (<14 lymphocytes and macrophages/mm²) the term viral cardiomyopathy or viral persistence in dilated cardiomyopathy should be applied.

The diagnosis of myocarditis and viral cardiomyopathy can be made only by endomyocardial biopsy, implementing the WHO/WHF criteria, and PCR techniques for identification of viral genome. The most frequent cardiotropic viruses detected by endomyocardial biopsy are Parvo B19, enteroviruses, adenoviruses, cytomegalovirus, and less frequently Epstein-Barr virus, and influenza virus.

Several studies have provided convincing support to the hypothesis that low-level expression of viral genome can induce chronic dynamic myocardial injury. Re-expression of human Coxsackie-adenovirus receptor (hCAR), as observed in dilated cardiomyopathy, may be a key determinant of cardiac susceptibility to viral infections. There may also be a familial predisposition for the development of inflammatory dilated cardiomyopathy. Out of the 208 patients, who met our criteria for inflammatory cardiomyopathy, 13 (6%) had family members with the same form of heart muscle disease.

In addition, humoral autoimmunity in postviral heart disease remains an attractive but controversial hypothesis addressing the pathophysiology of human viral cardiomyopathy. Antigenic mimicry with or without cytolytic antibody properties has been shown to play a role in the immunopathogenesis of myocarditis with respect to epitopes on sarcolemmal/myolemmal (including the beta-receptor) proteins, myosin and some mitochondrial proteins, including the ANT-carrier and dihydrolipoamid dehydrogenase.

Patients with dilated non-viral cardiomyopathy who deteriorate despite maximum medical management may be considered for immunosuppression. In patients with dilated cardiomyopathy and upregulated HLA expression, immunosuppression with prednisone and azathioprine significantly improved their left ventricular ejection fraction (LVEF) both at 3 months and 2 years after the initiation of treatment. Similar improvements were also evident at 2 years in NYHA functional class. Cardiac transplantation should only be considered when all other options, including mechanical circulatory assistance, have failed. Ongoing evaluation of antiviral therapies including the European Study of Epidemiology and Treatment of Cardiac Inflammatory Diseases (ESETCID), removal of antibodies by immunoabsorption, virus-specific vaccines, and mechanical support devices may provide new treatment options.

2. INTRODUCTION

The WHO/WHF Task Force on the Definition and Classification of Cardiomyopathies in 1995 established several changes in the terminology addressing cardiomyopathies (1). The term cardiomyopathy is no longer reserved for the idiopathic forms, but can be used interchangeably with the term heart muscle diseases. Right ventricular cardiomyopathy, valvular, hypertensive, ischemic or inflammatory cardiomyopathy have been introduced for the first time as cardiomyopathies. The new definition of the hemodynamically identified group of dilated cardiomyopathies also comprises INFLAMMATORY CARDIOMYOPATHY, defined as ”myocarditis in association with cardiac dysfunction”. Idiopathic, autoimmune, and INFECTIOUS forms of inflammatory cardiomyopathy were recognized.

VIRAL CARDIOMYOPATHY is defined as viral persistence in a dilated heart. It may be accompanied by myocardial inflammation and then termed inflammatory viral cardiomyopathy (or viral myocarditis with cardiomegaly). If no inflammation is observed in the biopsy of a dilated heart (<14 lymphocytes and macrophages/mm²), the term viral cardiomyopathy or viral persistence in dilated cardiomyopathy should be applied.

The identification and treatment of patients with viral cardiomyopathy is a major clinical challenge in modern cardiovascular medicine. The endomyocardial biopsy studies have led to the observation that patients with unexplained heart failure may have active inflammation. The treatment of this population remains a matter of debate. This review deals with our present knowledge on the viral cardiomyopathy in humans, analyzing in detail the mechanisms of cellular and humoral immune response during and after viral infection of the heart.

3. ETIOLOGIC AGENTS

The spectrum of the infectious agents that could be involved in the viral cardiomyopathy varies with the geographical region, the age of the patient, application of different therapeutic procedures, and additional diseases (Table 1). Numerous viruses may be associated with initial myocardial infection and further development of cardiomyopathy (2), but the Parvo B19, enteroviruses, and adenoviruses represent the most commonly identified agents. It is, however, impossible to determine the precise frequency in which cardiotropic viral infection results in clinically significant myocarditis and cardiomyopathy. Such information would require tissue sampling, from otherwise healthy subjects, during a viral epidemic.

3.1. Coxsackievirus

Both Coxsackie viruses A and B may produce myocarditis, although infection with Coxsackie B (CVB) is more common (3-6). At least 50% of healthy adults have detectable serum antibodies to CVB indicating prior infection (7-9). Using polymerase chain reaction (PCR), Fujioka *et al.* (10) have demonstrated plus-strand enteroviral RNA in 9/26 (35%) patients with DCM. Minus-strand enteroviral RNA was determined in 7/9 (78%) plus-strand RNA-positive patients. Sequence analysis revealed that the enteroviruses detected were Coxsackie B viruses, such as Coxsackievirus B3 and B4. Coxsackie A virus

Table 1. Viral etiology of myocarditis and DCM (review of the literature and Marburg myocarditis registry)(325, 326)

Virus order	Type	% positive in myocarditis	% positive in DCM
Picornavirus (RNA)	Coxsackie A and B virus	5-50	5-50
	Echovirus	?	?
	Hepatitis A virus	?	?
	Hepatitis C virus	0-15	0-10
Orthomyxovirus (RNA)	Influenza A, B	?	?
Paramyxovirus (RNA)	RSV, mumps virus, measles virus	?	<1
Rubivirus/Togavirus (RNA)	Rubella virus	?	<1
Rhabdovirus (RNA)	Rabies virus	?	?
Arbovirus/Tahyna (RNA)	Dengue, Yellow fever virus	?	?
Retrovirus/Lenti (RNA)	HIV (reversed transcriptase)	Variable	?
Herpesvirus (DNA)	Varicella zoster virus	1-2	1-2
	Cytomegalovirus	1-15	1-10
	Epstein-Barr-virus	1-3	1-3
	Herpes humanus 6 virus	0-5	0-5
	Herpes simplex virus	0-3	
Mastadenovirus (DNA)	Adenovirus	5-20	10-12
Parvovirus (DNA)	Parvo B 19 virus	10-30	10-25

DCM – Dilated cardiomyopathy

infections, classically affect more frequently the pleura and the pericardium (Bornholm diseases). In our registry, incidence of enteroviral myocarditis is 3%, of enteroviral cardiomyopathy with inflammation, 4% and, without inflammation, 4%.

In patients with enteroviral cardiomyopathy, necropsy often demonstrates a pericardial effusion, pericarditis, cardiac enlargement, and a predominantly mononuclear inflammatory infiltrate, with necrosis of the atrial and ventricular myocardium. In some cases, focal myocardial necrosis simulating myocardial infarction is seen, despite normal coronary arteries (11).

The myocardium appears to be particularly susceptible to the effects of enterovirus because of the affinity of myocardial membrane receptors for the viral particles. In the recent study, Noutsias *et al.* (12) have found the human Coxsackie-adenovirus receptor (hCAR) colocalized with integrins alpha(v)beta(3) and alpha(v)beta(5) on the cardiomyocyte sarcolemma and upregulated in DCM. This study suggested that low hCAR abundance may render normal human myocardium resistant to CAR-dependent viruses, whereas re-expression of hCAR, such as that observed in DCM, may be a key determinant of cardiac susceptibility to viral infections. Asymmetric expression of hCAR in the vessel wall may be an important determinant for adenovirus tropism in humans.

These findings are supported by clinical observations from our center, that there might be a familial predisposition for the development of inflammatory DCM. Out of the 208 patients, who met our criteria for inflammatory cardiomyopathy, 13 (6%) had family members with the same form of DCM (13).

3.2. Cytomegalovirus

Unrecognized infection with cytomegalovirus (CMV) is common in childhood, and the majority of the adult population has antibodies to CMV (14). Primary infection after the age of 35 years is uncommon, and generalized infection usually occurs only in immunosuppressed patients (15-17). The cardiovascular manifestations in adults are generally limited to asymptomatic and transient electrocardiographic abnormalities. Symptomatic cardiac involvement is rare, although a hemorrhagic pericardial effusion, or myocarditis with left ventricular dysfunction and attendant congestive heart failure may occur (16-18). In our registry, CMV-associated myocarditis and DCM were detected in <3% of the respective patient cohort.

3.3. Hepatitis

Clinical cardiac involvement in hepatitis is rare. However, there are contested data implicating hepatitis virus C (HCV) infection as an etiological factor in at least some cases of human viral cardiomyopathy (19). Fulminant myocarditis with congestive heart failure, hypotension, and death may occur in rare cases (20, 21). Myocardial damage may be produced indirectly through an immune-mediated mechanism or directly by viral invasion of the heart. The characteristic pathological changes in the myocardium associated with an infection with HCV are minute foci of necrosis of isolated muscle bundles, often surrounded by lymphocytes and a diffuse serous inflammation (21). The ventricles may be dilated, with petechial hemorrhages. Among 106 hearts examined by Matsumori *et al.* (19), HCV RNA was detected in 21.3%, and negative strands in 6.6%. HCV RNA was found in myocarditis in 33.3%, in DCM in 11.5%, and in hypertrophic cardiomyopathy in 26.0% of the patients. However, HCV RNA was not found in any of the patients with myocardial infarction or non-cardiac disease. In contrast to the Japanese data, incidence of HCV associated myocardial disease appear to be much less frequent in European patients ($\leq$1%).

3.4. Human immunodeficiency virus (HIV)

Cardiac involvement occurs in 25-50% of HIV

infected patients (22-24), with clinically apparent heart disease in approximately 10% (25-30). Congestive heart failure, due to left ventricular dilatation and dysfunction, is the most common finding (31, 32). Barbaro et al. (33) have investigated endomyocardial biopsy specimens from 82 HIV-DCM and 80 idiopathic DCM patients for determination of the immunostaining intensity of tumor necrosis factor (TNF)-alpha and inducible nitric oxide synthetase (iNOS) and for virological examination. Negative controls were derived from autopsy myocardium specimens from 32 HIV-negative patients without known heart disease. The mean intensity of both TNF-alpha and iNOS staining was greater in patients with HIV-DCM (0.81 and 1.007, respectively) than in patients with idiopathic DCM (0.44 and 0.49, respectively) or controls (0.025 and 0.027, respectively). The staining intensity of both TNF-alpha and iNOS inversely correlated with CD4 count. The staining intensity of iNOS was greater in HIV-DCM patients with HIV/CVB3 or with HIV/cytomegalovirus coinfection than in idiopathic DCM patients showing infection with CVB3 and adenovirus alone. The staining intensity of iNOS correlated to mortality rate (higher in HIV-DCM patients, in particular, in those with an optical density unit >1).

3.5. Infectious mononucleosis

Evident cardiac involvement in infectious mononucleosis is extremely rare, although nonspecific ST-segment and T-wave abnormalities may be seen. In rare cases, pericarditis and myocarditis (even simulating a myocardial infarction) may occur (34).

3.6. Influenza

Although clinically apparent myocarditis is rare in influenza, the presence of preexisting cardiovascular disease greatly increases the risk of morbidity and mortality (35). During epidemics, 5 to 10% of infected patients may experience cardiac symptoms (36). Postmortem findings in fatal cases include biventricular dilatation (37), with evidence of a mononuclear infiltrate (38), especially in perivascular areas. The prevalence of Influenza A, B, and C IgG antibodies is high in patients with DCM (39), but positive PCR findings in endomyocardial biopsies are very rare (<0.5% in our registry).

3.7. Mumps

Myocardial involvement during the course of mumps is rarely recognized (40, 41). Histologically, there is diffuse interstitial fibrosis, with infiltration of mononuclear cells and areas of focal necrosis (40). In our registry, incidence of PCR positive endomyocardial biopsies was <0.01%.

3.8. Poliomyelitis

Myocarditis occurs in about 5-10% of the cases during epidemics and is a frequent finding in fatal cases of poliomyelitis, occurring in half or more of all patients dying with this disease (42). Death may be sudden. Fortunately, this disease has been largely eliminated by immunization.

3.9. Respiratory syncytial virus

Although respiratory syncytial virus is an important cause of respiratory disease, particularly in children, it rarely results in cardiac involvement (43). Congestive heart failure and complete heart block have been seen on occasion (44).

3.10. Rubella

Rare cases of postgestational myocarditis occur, with attendant conduction defects and heart failure (45, 46).

3.11. Varicella

Clinical myocarditis is a rare finding in varicella, although unsuspected myocarditis is common in fatal varicella (47). Histological findings include rare but characteristic intranuclear inclusion bodies within the myocardial cells, along with interstitial edema, cellular infiltrates, and myonecrosis (48). We have observed only two biopsy-proven cases out of 3500 biopsied patients.

3.12. Variola and Vaccinia

Cardiac involvement following smallpox is rare, although several cases of myocarditis associated with acute cardiac failure and death have been reported. Myocarditis with pericardial effusion and congestive heart failure has also been observed as a complication of smallpox vaccination (49). Dramatic responses to steroids have been reported. The histological changes include a mixed mononuclear infiltrate, with interstitial edema and occasional degenerating or necrotic muscle bundles (50).

3.13. Adenovirus

Adenoviruses account for 3-5% of acute respiratory infections in children, but <2% of respiratory illnesses in civilian adults (51). Nearly 100% of adults have serum antibody to multiple serotypes. Infections occur throughout the year, but are most common from fall to spring. This is the second most frequent virus demonstrated by PCR in endomyocardial biopsies of patients with viral cardiomyopathy (51). In our registry, incidence of positive PCRs in patients with myocarditis and DCM was 5-8%.

3.14. Parvo B19

Infects most humans early in life without any major clinical sequelae. Significant disease only develops in people with an inappropriate immune response to the virus. It was recently recognized that Parvovirus B19 can cause myocarditis and either latent or active viral cardiomyopathy with high virus copy numbers in endomyocardial biopsies. Mean number of viral copies detected in patients with Dilated Inflammatory Cardiomyopathy (DCMI) was 2013, in comparison to 57 copies detected in DCM and 44 copies detected in hypertrophic cardiomyopathy (52). In a recent PCR series, Parvo B19 has been observed in up to 30% of investigated endomyocardial biopsy samples of patients with DCM and myocarditis (52, 53).

4. PATHOGENESIS OF MYOCARDIAL INJURY IN VIRAL AND POSTVIRAL MYOCARDITIS

Historically, the investigation of the immunological effector and modulator mechanisms in cardiomyopathies began with the demonstration of infiltrating lymphocytes in endomyocardial biopsies or at necropsy (reviewed in 54),

and continued with demonstration of antibodies against different cardiac tissue components, whose debated relevance was partly defined by in vitro or in vivo experiments (reviewed in 55). Organ-specificity, cross-reactivity, and antigenic mimicry are issues still at stake, particularly after wide application of PCR techniques demonstrated viral genomes in myocardial tissue specimens (reviewed in 56). The studies elaborating expression of cytokines and adhesion molecules, as well as their effect on myocytes, endothelial cells, and fibroblasts support the involvement of humoral factors involvement in the pathogenesis of cardiomyopathy. In addition, several studies have revealed both T-cell-immune mediated and virus-induced cardiac damage as the major pathophysiological mechanisms (reviewed in 57 and 58). Nevertheless, apoptotic cell death may provide another concept to explain a harmful clinical course of acute myocarditis (59). At present, a complex concept of the pathogenesis has evolved and reached consensus "in principle".

4. 1. The Postviral Autoimmunity Hypothesis

According to this hypothesis, the initial viral infection of the myocardium produces limited myocardial lesions but triggers an adverse immune response that is primarily responsible for myocyte damage and resultant heart failure (54). Viral infection causes initial myocyte damage, releasing myosin into the circulation. The viral infection then clears, but is followed by a second phase of disease mediated largely by antibodies to myosin heavy chain and CD4+ T lymphocytes. The latter may produce myocyte damage not only by inducing B cells to produce antimyosin antibodies but also by stimulating cytokine accumulation and by inciting production of cytotoxic CD8+ T lymphocytes. One to several weeks delay between recovery from a viral infection and development of heart failure is commonly reported.

4.2. Antigenic Mimicry Hypothesis

The infectious agent carries an antigen identical or similar to normal myocyte antigens (5). The antibody response to the antigen cross-reacts with the infecting agent and the myocyte (60). Cellular immune responses also may be induced by cross-reactive epitopes. Antigenic mimicry has therefore evolved as an important pathomechanism by which sensitized T-cells and autoantibodies could cause cardiac damage independent from the viral infection of the heart itself (61). In humans, cytolytic antibodies against the myolemma and sarcolemma of isolated myocytes that cross-react with enteroviral proteins have been demonstrated. By absorption experiments with either the virus or a human heart membrane extract or a pellet of isolated myocytes the antibody immunofluorescence on isolated human and rat myocytes vanished. Consequently the cytolytic properties of absorbed sera, which previously required the presence of complement to work, were completely abolished.

4.3. Direct Viral Injury Hypothesis

After successful replication within the myocyte, highly lytic viruses may destroy the host cell. This damage can occur in the complete absence of host immune defenses, and viral proliferation alone is then sufficient to initiate severe myocarditis with resultant heart failure (62).

4.4. Direct Immune Injury Hypothesis

Less lytic viruses may continue to infect the cell without killing it, but nevertheless cause its destruction by inducing a virus-specific immune response. Nonspecific responses may also be involved (nitric oxide, cytokines)(63, 64).

5. HUMORAL IMMUNE MECHANISMS

5.1. Myocardial Antigens in Autoreactive and Postviral Heart Disease

Various cardiac antigens can be identified as targets of humoral and cellular autoreactivity. They include components to be identified by light microscopy on cryostat sections or antigens characterized biochemically or defined by monoclonal antibodies. A tabulation of the respective organ-specific- and non-organ-specific-antibodies is presented in Table 2. Myolemma and sarcolemma are antigens with different subepitopes have been extensively analyzed in our laboratory (55, 56, 61, 65-82). Various receptors such as the Ach-receptor, the β1-receptor and the Ca^{2+} channel have been studied by others (Table 3), as well as contractile and other intracellular or extracellular proteins (myosin, actin, laminin, vimentin, desmin) and enzymes (aconitate hydratase, pyruvate kinase, dihydrolipoamide dehydrogenase, creatine kinase, nicotinamideadenine dinucleotide dehydrogenase, ubiquinol-cytochrome-c reductase, adenine nucleotide translocator), carnitin, heat shock proteins, nuclear antigens, extracellular matrix, and endothelial cell antigens. All these molecules/structures have been either postulated to be involved in an antigenic mimicry process or associated with the major cardiotropic viruses for a possible cross-reactivity with viral antigens. However, except for the sarcolemmal/myolemmal, myosin epitopes, and some mitochondrial proteins, the evidence for cross-reactivity is either hypothetical or has yet to be reproduced (39).

5.2. Specific Antibodies to the Cardiac Membrane and its Constituents

5.2.1. Antimembrane Antibodies

Circulating and bound antibodies to the membrane of the cardiomyocyte, the sarcolemma and myolemma, which may be cytolytic and complement-fixing have been demonstrated in Coxsackie virus B, mumps, and influenza myocarditis (61, 68, 70-73, 83). Epitopes on the sarcolemmal surface (69) were found to cross-react with epitopes on Coxsackie B viruses. In absorption experiments it could be shown that in viral myocarditis, the sarcolemmal fluorescence was greatly diminished, and the cytolytic serum activity could be absorbed by the respective viruses. This could be done for Coxsackie B and influenza virus (55, 68,72, 74, 76). In addition, titer of antimyolemmal antibodies and cytolytic serum activity correlated nicely, demonstrating the presence of complement-fixing cytolytic antimembrane antibodies. It could be also shown that they are cross-reactive to enteroviral core proteins by western blot analysis (69). The cytolytic property of the patients' sera in vitro suggests, in contrast to the recent findings of Horwitz *et al.* (84), that humoral autoreactivity and

Table 2. Proportion of patients with myocarditis or dilated cardiomyopathy with circulating antibodies to the sarcolemma, extracellular matrix, and intermediate filaments

Author (Reference):	N	AMLA (homol.)	ASA (homol.)	ALA	Z-bands	A-Actin	A-Myosin	A-Tubulin	AIDA	A-M7	AEA	A-Collagen type			
												I	II	III	IV
De Scheerder (327)	12	100%	12%			58%	67%				91%	35 %	40 %	35 %	35 %
Klein (90, 91)										13%/30 %*					
Maisch (68, 72, 76, 77)	44/ 79*	79-90%/ 9%*	10%*		15%	7%/4%*	10-50%/ 20%*	0	0%/2%*		80%/13% *				
Maisch (73)	30*	33%*	42%*			10%*	33%*		2%*		45%*				
Idiopathic myocarditis (56)	144	59%	45%			0	23%	9%	0		40%				
Myocarditis in children (81)	43	100%	100%		<1%	<1%	<1%	<1%	<1%		91%				
Maisch (75)	132			30-35%											
Obermayer (114)	25/ 36*	64%/42 %*	72%/ 31%*		16%/ 8%*	0/0*	4%/8%*	0			72%/31% *				
Schultheiss (93, 95, 96)	29/ 51*			60%/ 72%*								35 %/ 12 %*	40 %/ 24 %*	35 %/ 6% *	35 %/ 24 %*

* findings in patients with dilated cardiomyopathy (% of the patients positive) are marked with an asterix (*) in contrast to the findings in patients with myocarditis with no asterix; AMLA – anti-myolemmal antibody; ASA – anti-sarcolemmal antibody; ALA – anti-laminin antibody; A – anti; AIDA – anti-intercalated disc antibody; AEA – anti-endothelial antibody

antigenic mimicry are major pathogenetic principles operative in human enteroviral myocarditis and its sequelae.

Presence of antibodies to the **beta adrenoceptor (BAR)** has been shown in 30-70% of patients with DCM (85, 86). These antibodies appear to have negative inotropic and chronotropic effects, similar to beta blockers. In contrast, beta-receptor antibodies that increase the beating frequency of isolated fetal heart cells have also been demonstrated (87). These antibodies induce an Mg^{2+}-dependent conformational change to the receptor, independent of coupling to the GTP regulatory protein, but similar to that induced by the agonist isoproterenol (88).

5.2.2. Bound Antimembrane Antibodies in Endomyocardial Biopsy Specimens

Antimembrane antibodies not only circulate in the peripheral blood, but are also bound to the sarcolemma and the interstitial tissue (72, 76, 77, 89). In a multicenter study (76), IgG fixation was found in more than 80% of patients with myocarditis and inflammatory DCM. IgM, IgA, and C3 or C1q fixation, were of particular diagnostic value, indicating secondary, post-inflammatory immunopathogenesis.

5.3. Antibodies to Intracellular Antigens

5.3.1. Antimitochondrial Antibodies

Antibodies to **mitochondrial proteins** have been demonstrated both in patients with myocarditis and DCM. They are specifically developed against the *M7 protein* (90, 91) and its relevant constituent sarcosin dehydrogenase (92) and the *adeninenucleotide translocator (ANT)*(93, 94-98).

The **ADP/ATP carrier protein** is responsible for the transport of high-energy phosphates across cell (mitochondrial and other) membranes. Antibodies to the carrier have been identified in humans, as well as in animal models (94, 99, 100). These antibodies have been demonstrated to decrease the external work of isolated, perfused, spontaneously beating hearts (101). ADP/ATP carrier antibodies from patients with myocarditis and DCM enhanced calcium influx by increasing the calcium current.

Anti-ANT antibodies have also been shown to interfere with the energy metabolism of the myocardial cells. It has been suggested (102, 103) that the ANT antibodies may cross-react with calcium channel proteins. In animal models of CVB3 infection, Schultheiss *et al.* (95) reported an inhibition of nucleotide transport and reductions in stroke volume and work in animals with anti-ANT antibodies in contrast to controls. By using synthetic peptides, cross-reactivity between peptides from the adenine nucleotide translocator of the inner mitochondrial membrane and peptides from Coxsackie virus B3 has been demonstrated (104). There are two isoforms of ANT. An increase in the ANT1 isoform was detected in patients with enterovirus infection with a concomitant decrease in ANT2.

5.3.2. Antisarcoplasmic Antibodies

Using an experimental myocarditis model Khaw *et al.* (127) investigated the development of autoimmunity to cardiac sarcoplasmic reticulum calcium ATPase (**SR-Ca^{2+} ATPase**). Immunization of CAF1/J mice with affinity-column-purified canine cardiac SR-Ca^{2+} ATPase produced a time-dependent induction of myocarditis. Furthermore, the antibody used in affinity purification of the ATP-ase (4C11-20.21) can alone induce myonecrosis in severe combined immunodeficiency (SCID) mice, indicating a mechanism of cardiomyopathy independent of the cytotoxic T-cell mediated autoimmunopathy.

5.3.3. Antimicrosomal Antibodies

An antigen-specific immune response to cardiac epitopes was demonstrated by our group (83) in 73% of 54

Table 3. Compilation of antibodies to cardiac antigen and their possible cross-reactivity and pathomechanism (in alphabetical order)

Antigen	Antibody	Cross-reactivity with *experimentally proven ? hypothetical	Pathomechanism * experimentally proven ? hypothetical	Author (Reference)
Actin	Anti-actin	Unknown	Unknown	Maisch 1993 (69)
Ach-receptor	Anti-Ach	Unknown	Bradycardia?	Goin 1999 (326)
AH, PK, DLD CK	Anti-AH, Anti-PK, Anti-DLD, Anti-CK	Unknown	Impairment of energy metabolism?	Pankuweit 1997 (83)
ANT	Anti-ANT	Enterovirus?	Impairment of mitochondrial energy metabolism and/or disorder of the permeability transition	Schulze and Schultheiss 1995 (328)
Beta1-receptor	Anti-beta1	Enterovirus?	Positive chronotropic *	Wallukat 1995 (87)
Beta1-receptor	Anti-beta1		Negative inotropic?	Limas 1990 (329)
Ca^{2+} channel	Anti-Ca^{2+} channel proteins	ANT? Enterovirus?	Unknown	Schulze 1999 (330)
Carnitin	Anti-carnitin		Unknown	Otto 1999 (331)
Conduction system	Anti-sinus Anti-AV node Anti-Purkinje	Unknown	Conduction defect?	Maisch 1986 (79)
Desmin	Anti-desmin		Unknown	Maisch 1987 & 1989 (67, 75) Obermayer (114)
Hsp60, hsp70, Vimentin	Anti-hsp60, Anti-hsp70, Anti-vimentin	Multiple	Unknown	Portig 1998 (180)
Laminin	Anti-laminin		Unknown	Maisch 1991 (80)
Mitochondria /Microsoms	AMA	Multiple*	Inhibition of sarcosin dehydrogenase, a mitochondrial key enzyme or ?	Klein 1984 (90), Pohlner 1997 (84)
Myolemma	AMLA	Enterovirus *	Lytic *	Maisch 1993 (69)
Myosin	Anti-myosin		Negative inotropic?	Maisch 1987 (56)
Myosin	Anti-myosin	Enterovirus?	Negative inotropic?	Caforio 1996 (332)
NADD UCR	Anti-NADD Anti-UCR	Unknown	Impairment of energy metabolism?	Pohlner 1997 (92)
Nuclear antigen ENA	ANA Anti-ENA ANCA Anti-SSA Anti-SSB	Unknown	Immune complex – mediated Degranulation of neutrophils? AV-Block	Naparstek 1993 (333)
Sarcolemma	ASA	Enterovirus *	Lytic *	Maisch 1993 (69)
SR-Ca-ATPase			Metabolic interactions interactions on second messanger level and at the sarcoplasmic reticulum and/or alterations in the beta-adrenergic pathway	Khaw 1995 (334)

Abbreviations: Ach – acetylcholin, AH – aconitate hydratase, AMA – antimytochondrial antibody, AMLA – antimyolemmal antibody, ANA –antinuclear antibody, ANCA – anti-neutrophil cytoplasmic antbodies, ANT – adenine nucleotide translocator, ASA – antisarcolemmal antibody, CK – creatine kinase, DLD - dihydrolipoamide dehydrogenase, ENA – extractable nuclear antigen, hsp – heat shock protein, NADD – nicotinamideadenine dinucleotide dehydrogenase, UCR – ubiquinol-cytochrome-c reductase, PK – pyruvate kinase, SR-Ca-ATPase – sarcoplasmatic reticulum calcium ATP-ase.

patients with histologically proven myocarditis, utilizing the indirect immunofluorescence test with human myocardium and adult heterologous cardiocytes. By immunoblot, 44% of the sera reacted with cardiac tissue. These antibodies were directed preferentially against proteins with a molecular weight range of 43 to 67 kD. One of these proteins was found to be **dihydrolipoamide dehydrogenase** and the other was identified as a **sarcomere-specific creatine kinase**.

5.4. Antibodies to Fibrils

Antibodies to **fibrils**, particularly antibodies to **myosin** and **actin**, have been reported in human (105) and murine myocarditis (106). In a large series, Caforio *et al.* (107) described cardiac antimyosin autoantibodies in 25-35% of patients with myocarditis and DCM. The frequency of organ-specific cardiac autoantibodies was significantly higher in myocarditis (45%) and in DCM (20%) than in other cardiac diseases (1%), or in normals (2.5%). Myosin antibodies were also detected in family members of patients with enteroviral heart disease. This might point out to an infectious pathway via cross-reactive antibodies, but may also indicate a familial predisposition to antibody production of autoreactive postviral heart disease. Myocardial imaging using an indium-111 labeled antimyosin monoclonal antibody was established for the assessment of the disease activity and extent of myocardial damage in patients with myocarditis and DCM (108). Lauer *et al.* (109) found antimyosin autoantibodies in 17/33 (52%) patients with chronic myocarditis, proven on endomyocardial biopsy at the initial presentation. After six months, antimyosin autoantibodies were still found in 13 (76%) initially antibody-positive patients. No initially antibody-negative (n=16) patients developed antimyosin autoantibodies during follow-up. Clinical symptoms improved slightly in antibody-negative patients and remained stable in antibody-positive patients. Importantly, left ventricular ejection fraction recovered significantly better in antibody-negative patients (+8.9±10.1%) compared with antibody-positive patients (-0.1±9.4%).

Other antibodies directed to **antigenic intracellular enzymes such as branched chain alpha-keto-acid dehydrogenase** have also been identified (110). Antibody-mediated interference with metabolic activity without alteration of cell viability is a plausible explanation for reversible myocardial dysfunction following myocarditis.

5.5. Antibodies to the Extracellular Matrix

Further, non-organ-specific but defined antigens include **desmin** (in myocytes), **vimentin** (marker of fibroblasts and histiocytes), **collagen**, **laminin** (111), and **fibronectin** (67).

Vimentin autoantibodies arise in the murine-encephalomyocarditis virus (EMC) model of myocarditis within 9 days after initial infection (112). When an extract of cardiac C-protein was used to immunize syngenic mice, autoimmune myocarditis characterized by severe inflammation resulted (113). Ubiquitous antigens such as the micro- and intermediate filaments, the macrofilaments, or the extracellular matrix constituents, may evoke non-organ-specific immune responses, in contrast to species-specific or even individually unique epitopes, such as the major histocompatibility complex constituents. The tissue-specific epitopes could to be of greater importance in immune diseases restricted to the myocardium. Antibodies to the extracellular matrix components occur frequently in endomyocardial biopsies (67, 68, 102) and less frequently in the serum of patients with myocarditis (114).

5.6. Antiendothelial Antibodies

Antiendothelial antibodies, which may be cytolytic to living cultured human endothelial cells, were demonstrated in myocarditis (115, 116). These antibodies can also be demonstrated in endomyocardial biopsy specimens of patients with biopsy-proven myocarditis.

5.7. Circulating Immune Complexes

Immune complexes composed of a soluble antigen and specific antibody are formed in the circulation and may deposit in vessel walls anywhere in the body. This leads to local activation of leukocytes and the complement system, with resultant tissue injury. In myocarditis, circulating immune complexes may be present at the time of the myocarditic viral illness in the majority of patients (117). They may be in part responsible for some of the systemic features during this viral illness e.g. proteinuria or erythrocyteuria and even myalgia. Immune complex deposition can be also seen in endomyocardial biopsy specimens. In patients with inflammatory DCM immune complexes have been found more frequently than in normals and in patients with DCM, without inflammation (78). These circulating immune complexes consisted of IgG, IgM, C3 and C4, but in myocarditis, IgM predominated (118). Up to now, the nature of the soluble antigen has not been determined (foreign or self?).

6. CELLULAR EFFECTOR MECHANISMS

Humoral immune mechanisms, although intriguing in selected models, do not fully explain the abnormalities detected in viral cardiomyopathy, and likely the immunologic mechanisms resulting in myocardial injury are complex and multifactorial (119). Cellular immunity may play an important role by direct mediation of myocyte injury, immunoregulation, or generation of cytokines.

When weanling mice were inoculated with CVB3, viral replication was completely suppressed within one week (120). After viral clearance, a cellular infiltrate appears, marking the immune phase. Mice who received rabbit anti-thymocyte serum or were thymectomized, lethally irradiated and had bone marrow reconstitution prior to infection with CVB3 were capable of clearing the virus normally despite their lack of cellular immunity. However, the chronic inflammatory infiltrate was attenuated. These studies emphasize the importance of cell-mediated immunity in the chronic phase of the disease. Autoreactive cytotoxic T-lymphocytes, which adsorbed to and lysed both infected and uninfected myocytes caused extensive

necrotizing lesions (121). Cytotoxic T-lymphocytes that only lysed viral-infected cells were also generated. When lymphocytes were cultured from infected murine hearts, the predominant cells were T-cells (alloreactive cytotoxic subset) (122).

Perforin, the cytolytic factor expressed by T-lymphocytes, is found in the cytoplasmic granules of cells infiltrating the myocardium, suggesting direct cytotoxic myocyte death (123, 124). Immunohistochemical studies from hearts of patients with acute myocarditis could identify perforin in the infiltrate (125), although it remains unclear whether this mechanism of cell death is the most important one. Apoptosis and necrosis by the virus or immune cells appear to be equally significant.

6.1. Adhesion Molecules

In DCM with and without inflammation, the following adhesion molecules, responsible for leukocyte adhesion to resting or cytokine-activated endothelium and mediation of inflammatory reactions, have been studied: E-selectin, E-selectin ligand, LFA-1 (lymphocyte function-associated glycoprotein), VLA-4 (very late activation antigen), ICAM-1, -2, and -3 (intercellular adhesion molecule), and VCAM-1 (vascular cell adhesion molecule). Inflammatory endothelium activation is present in a large percentage of patients with DCM. A correlation was demonstrated between the expression of cell adhesion molecules, the immunohistological diagnosis of inflammatory DCM, and counterreceptor-bearing intramyocardial infiltrates (126, 127). The mechanisms leading to the expression of cytokine-induced cell adhesion molecules on endothelium in patients with DCM preceding the inflammatory response are still not fully understood. In a mouse model of myosin-induced myocarditis, the expression of class II MHC and/or endothelial ICAM-1 is a prerequisite for emigration of myosin-reactive T cells (128).

Sensitized cytotoxic T-lymphocytes produce myocardial injury that is calcium and protein kinase C-dependent in vitro, resulting in contractile abnormalities in beating heart cells (129). T-lymphocytes may modify these cellular responses via cytokines. Serum from patients with myocarditis and DCM demonstrate an increase in ICAM-1, IL1-a, IL 1-b, tumor necrosis factor-a (TNF), and macrophage stimulating factor (130, 131). Cell interactions and adhesion are necessary to induce immune-mediated myocardial injury, underscoring the importance of ICAM-1 expression (132).

6.2. Natural Killer Cell Activity

Natural killer (NK) cell activity in patients with myocarditis and perimyocarditis is markedly decreased in the acute stage. In postmyocarditic dilated muscle heart disease, NK cell activity returns to normal . In primary DCM however, a significantly decreased NK-cell activity can be observed again (133, 134).These
functional data do not necessarily substantiate the perforin data cited above.

6.3. Target Cell Specific Non-MHC Restricted Lymphocytotoxicity

In myocarditis, target cell specific non-MHC restricted lysis of living adult allogenic rat myocytes by circulating patients` lymphocytes is sustained or slightly enhanced. This also applies to postmyocarditic dilated heart disease and primary DCM in which one third of patients demonstrated an increase of target cells-specific cytotoxicity. Analysis of antibody-dependent cellular cytotoxicity showed little variation from normal (72).

6.4. Cytokines

Cytokines may induce or exacerbate myocarditis through several mechanisms. They may activate cytotoxic T cells and induce expression of cell adhesion molecules and iNOS. Nitric oxide and some cytokines can directly damage myocytes and cause reversible depression of contractility. Cytokines mediate activation and the effector phases of innate and specific immunity, which are both important in controlling a viral infection. The innate immune response not only has an important protective function, but also serves to initiate and regulate subsequent specific immune responses (57). There are two principal mechanisms of **innate immunity** against viruses:

1. Viral infection directly stimulates the production of type I IFN (IFN alpha and beta) by infected cells. Type I IFN inhibits viral replication by initiating the synthesis of a number of enzymes, which collectively interfere with replication of viral RNA or DNA.

2. NK cells lyse a wide variety of virally infected cells, and are probably one of the principal mechanisms of immunity against viruses early in the course of infection, before specific immune responses develop (135, 136).

In **specific immunity,** various cytokines, chemokines, and adhesion molecules are involved in regulating migration and activation of T- and B-cell responses including migration and activity of macrophages. Elevated levels of TNF-alpha, IL-1, and IL-6 have been reported in plasma of patients with myocarditis. TNF-alpha is able to potentiate the immune response and induce apoptosis in cells, both of which appear to hold special importance in the pathogenesis of myocarditis. Other inflammatory mediators, including interleukin-1 (IL-1) and granulocyte colony-stimulating factor (GM-CSF), are also elevated in sera of myocarditis patients. Moreover, plasma levels of TNF-alpha and IL-6 correlate with clinical signs of heart failure in patients with DCM (108).

One possible effect of cytokine expression is the activation of iNOS, which may have beneficial effects because of the antiviral activity of nitric oxide (137, 138). On the other hand, excessive nitric oxide production may be detrimental, causing myocardial depression, apoptosis, and necrosis (139, 140). Increased expression of iNOS has been proposed to account for some of the dilation associated with DCM (141) and has been demonstrated in a murine CVB3-induced myocarditis model (142). However, myocarditis develops also in mice lacking interferon regulatory transcription factor (IRF)-l, a transcription factor that controls iNOS expression. In contrast to these data, Ishiyama *et al.* (139) found that nitric oxide expression played a critical role in the resultant pathology produced in

a rat model of autoimmune myocarditis, after induction with cardiac myosin. Rats that were treated with aminoguanidine (iNOS inhibitor), had only focal mononuclear infiltration and reduced numbers of cardiomyocytes positive for iNOS. In addition, serum levels of creatine kinase were significantly reduced in the treated animals, indicating reduced muscle damage.

Reports from several laboratories demonstrating that TNF-alpha exacerbates myocardial injury (143-145) have contributed significantly to the understanding of the mechanisms of acute myocarditis. An increased intracellular concentration of cAMP, by stimulating the beta-adrenergic receptors, may accelerate the rate of cell death, and calcium overload may induce arrhythmias and myocardial injury (146, 147). In addition, several studies have shown an integration of neuroendocrine hormones into the immune response and an increase in mortality associated with the long-term use of beta-agonists (148).

7. CLINICAL PRESENTATION

The clinical manifestations of viral cardiomyopathy span from an asymptomatic condition to progressive cardiac dysfunction or sudden unexpected death. An initial episode of myocarditis is most commonly recognized by an acute febrile syndrome, associated with pericardial and systemic complaints related to the viral infection (149). The cardiovascular complaints may be minimal, and the disease may be manifested only by an abnormal electrocardiogram (150). Most patients with this presentation will experience rapid resolution. One rather interesting manifestation of inflammatory heart disease is the presentation similar to acute myocardial infarction. This syndrome was initially described by historical, electrocardiographic, enzymatic, and radionuclide abnormalities (151-153). Echocardiography may confirm segmented wall motion abnormalities or aneurysm (11, 154). Most patients have complete recovery. However, isolated case reports of progressive myocardial cell loss and death have been reported (155, 156). The segmental infiltration of the myocardium most likely results from viral/immune mediated injury; however, coronary arteritis and vasospasm have also been implicated (157–159). Syncope due to Stokes-Adams attacks is a common manifestation, particularly in childhood (160). These episodes may result from atrial myocarditis presenting with sinoatrial block or atrial standstill, atrioventricular block, or intraventricular conduction abnormalities (161–165). Heart block is a particularly notable presentation with Epstein-Barr virus, mumps, or rickettsia infections (166–168). Ventricular arrhythmia as a precursor to sudden cardiac death is a particularly worrisome manifestation (169–171). Although not a very prominent cause of unexpected death in the overall population (172, 173), myocarditis accounts for 17-25% of unexplained sudden death in otherwise active, healthy young people (174, 175). Fulminant myocarditis is characterized by critical illness at presentation, but excellent long-term survival. In contrast, patients with acute myocarditis are less ill initially, but may have a progressive course that leads to death or the need for cardiac transplantation (176). Myocarditis may also mimic other forms of heart muscle disease, periodically presenting with increased left ventricular mass and diastolic dysfunction compatible with restrictive cardiomyopathy (177) or asymmetrical thickening and reduction in cavitary size resembling hypertrophic cardiomyopathy (178, 179). Finally, an inflammatory heart disease may cause unexplained congestive heart failure. The historical, clinical, laboratory, and echocardiographic manifestations may be indistinguishable from idiopathic DCM.

8. DIAGNOSTIC EVALUATION

8.1. Laboratory and Noninvasive Testing

In acute myocarditis, erythrocyte sedimentation rate and C reactive protein are increased in up to 60% of patients. Leykocytosis can be present in 25%, and a rise in creatine kinase is detected in 12% of the patients (58). Raised titres of antibodies against cardiotropic viruses may be present but simply denote infection at some time. A four-fold rise in IgG titre over a 3-4 week period is necessary to establish acute infection. However, such findings are not sufficient to establish the diagnosis of viral myocarditis.

Elevated anti-heart antibody titers are common in patients with cardiomyopathy and myocarditis (180-183). However, these anti-heart antibody preparations were not specific for the diagnosis of inflammatory heart disease (184). Enthusiasm was rekindled by the identification of antisarcolemma antibodies of the antimyolemma type that lysed vital adult rat cardiocytes, and were specific for inflammatory heart disease (72).

Electrocardiographic abnormalities are common in acute myocarditis, comprising sinus tachycardia, diffuse ST-T wave abnormalities, prolonged QT interval, conduction abnormalities, and supraventricular as well as ventricular tachyarrythmias (185, 186). Complete heart block may occur, but is usually transient and the implantation of a permanent pacemaker is rarely necessary (180).

In echocardiography, left ventricular dimensions are not usually increased in the acute phase. A characteristic trabeculated pattern of interventricular septum and ventricular walls may sometimes be found early in the course of the disease if inflammation is substantial. However, due to low sensitivity and specificity, the trabeculated pattern has not been particularly helpful for the diagnosis (187). However, echocardiography has confirmed that myocarditis may present not only as DCM but also with asynergic ventricular wall motion, increased left ventricular wall thickness, and mass or restrictive ventricular filling (188). When directly compared to a similar cohort of patients without active myocarditis on biopsy, left ventricular end systolic dimension index was smaller and fractional shortening greater in patients with myocarditis compared to those without (189). However, great overlap existed between the two patient populations making echocardiographic differentiation impossible. Mural thrombi have been noted in up to 15% of patients (190). An echocardiographically demonstrated pericardial

effusion in a dilated, segmentally asymmetric heart may indicate myopericarditis (3).

The advent of nuclear cardiology brought renewed interest in the noninvasive identification of active myocarditis. Technetium-99m-pyrophosphate, the infarct-avid radioisotope, localizes to the myocardium of mice previously infected with CVB3 (191). Although isolated case reports demonstrate utility in man (192), the low specificity prevented the broad application of this technique to clinical trials. The inflammation-avid radioisotope, gallium-67, demonstrated avidity to the inflamed myocardium in patients with DCM and biopsy-proven myocarditis (193). In expanded clinical trials, the sensitivity and specificity of this technique did not justify clinical use (194-197). Indium-111-monoclonal antimyosin antibody imaging is highly sensitive with low specificity for myocarditis (198). As is evident with gallium-67 imaging, nonspecific myocardial damage may also localize the isotope (199). When uptake of indium-111 antimyosin antibody was assessed in patients before heart transplantation and histology from the explanted hearts compared, active myocarditis was detected in >50% of those with uptake (200). Collectively, the noninvasive imaging techniques for the detection of inflammatory heart disease, although in theory quite promising, have not been sufficiently accurate to be considered as a substitute for myocardial biopsy or as a screening tool prior to biopsy.

Contrast-enhanced magnetic resonance imaging (201-204) and echocardiographic digital image processing (205) may also be useful for the non-invasive localization and assessment of the extent of inflammation in patients with presumed myocarditis. Their criteria are indirect, however. Interstitial swelling, edema, and wall-motion abnormalities detected by magnetic resonance imaging can be also identified in a "one stop shop". In echocardiography, including second harmonic imaging, segmental asynergy, contraction and relaxation abnormalities are assessed. Nevertheless, all of these criteria are only indirect evidence and not a true proof of myocardial inflammation.

In summary, although serologic markers of myocardial injury, imaging of the myocardium to detect inflammation or injury, and noninvasive cardiac testing have been helpful in ascertaining the extent of myocardial dysfunction in patients with myocarditis, these modalities can not establish the diagnosis, and endomyocardial biopsy remains the diagnostic standard.

8.2. Endomyocardial Biopsy

A successful, safe intravascular biopsy technique was developed by Konno and Sakakibara (206) and further improved by Richardson (207). Mason *et al.* (208) were first to report the utility of endomyocardial biopsy in establishing the diagnosis of myocarditis. Numerous reports documenting myocarditis in patients presenting with unexplained congestive heart failure followed (36, 208-240). Similar observations were made in patients with unexplained ventricular arrhythmia (241-248). However, it became apparent that there were wide discrepancies in pathologic interpretation of a single biopsy. The frequency of myocarditis in comparable heart failure populations varied from 0-67%. The diagnostic standards for active myocarditis varied among institutions (249). Some investigators established the diagnosis of myocarditis by quantitation of the infiltrate, specifically >10 lymphocytes per high power field (250). Others questioned this threshold and proposed "chronic" terminology for lesser degrees of infiltration (251). The WHF criteria today postulate a chronic myocarditis or DCM with inflammation when $\geq$ 14 lymphocytes/mm² are identified preferably by sensitive immunohistochemical methods (65).

Another issue in interpretation was the accuracy of identification of the mononuclear cells in the infiltrate by light microscopy (252-254) and the sampling error. Commonly documented segmental wall motion abnormalities imply that myocarditis may be focal or patchy. The analogy to cardiac transplantation in which sampling error was minimized by removing multiple biopsy samples led to recommendation to take at least four to six fragments for diagnosis (255, 256). Finally, the documentation of rapid histological resolution questioned the timing of endomyocardial biopsy (254).

However, endomyocardial biopsy has been useful in predicting morphologic progression in patients with active myocarditis. Serial sampling has shown convincingly that active myocarditis may progress to end-stage cardiomyopathy, as suggested by the epidemiologic studies performed decades earlier (257). Active myocarditis has been confirmed in families with cardiomyopathy and an immunologic predisposition for augmented immune responses following initial viral infection (258). Endomyocardial biopsy has confirmed active myocarditis in a high percentage of women with peripartal cardiomyopathy (259-263), and myocarditis due to human immunodeficiency virus (264).

Although virus could be isolated from stool or urine, virus isolation from pericardial fluid or the myocardium itself was rarely reported (265-270). Considering the extensive clinical effort to isolate virus from human tissue, viruses are almost never cultured from human myocardium in the immunocompetent host.

The application of molecular techniques to identify viral genomic fragments *in situ* has significantly increased the diagnostic value of endomyocardial biopsy (53, 270, 271)(Table 4). However, further analyses of enteroviral positivity as a determinant of prognosis in patients with myocarditis were conflicting. While Why *et al.* reported that myocardial enteroviral RNA portends a poor prognosis (271), Figulla *et al.* reported improved heart transplantation-free survival in enterovirus positive patients (272). We favor the hypothesis that a myocardial cell that contains active replicating virus is lost.

8.3. Evaluation of Endomyocardial Biopsy Findings

The "Dallas" criteria defined "active" myocarditis as "an inflammatory infiltrate of the myocardium with necrosis and/or degeneration of adjacent

Table 4. Polymerase chain reaction (PCR) findings in the endomyocardial biopsy specimens. Review of the literature

Virus	Diagnosis	PCR +	Author (reference)
Enteroviruses	DCM	9/26 (35%)	Fujioka 2000 (10)
	DCM	21/55 (38.2%)	Rey 2001 (335)
	DCM	9/21 (42.9%)	Archard 1998 (336)
	DCM	4/53 (7.5%)	Giacca 1994 (337)
	DCM	6/19 (31.6%)	Schwaiger 1993 (338)
	DCM	6/50 (12%)	Keeling 1992 (339)
	DCM	11/19 (57.9%)	Andreoletti 1996 (340)
	DCM	7/42 (16.7%)	Ueno 1995 (341)
	DCM	30/45 (66.7%)	Petitjean 1992 (342)
	Myocarditis	18/45 (40%)	Pauschinger 1999 (343)
	Myocarditis	8/38 (21%)	Martin 1994 (344)
	Myocarditis	4/5 (80%)	Ueno 1995 (341)
	Myocarditis	2/10 (20%)	Hilton 1993 (345)
	Myocarditis	5/6 (83.3%)	Nicholson 1995 (346)
	Controls (autopsies)	13/75 (17.3%)	Hilton 1993 (345)
Hepatitis C	DCM	3/36 (8.3%)	Matsumori 1995 (347)
	Myocarditis	4 (33.3%)	Matsumori 2000 (348)
	HCM	6/42 (26%)	Matsumori 2000 (349)
Parvo B19	DCM/Myocarditis	30%	Maisch, Pankuweit 2000 (53, 66)
	Susp. myocarditis	3/360 (0.8%)	Schowengerdt 1997 (350)
	Transplant reject.	6/200 (3%)	Schowengerdt 1997 (350)
Adenovirus	CHF	12/94 (12.8%)	Pauschinger 1999 (351)
	Myocarditis	15/38 (39.5%)	Martin 1994 (344)
	HIV	6/32 (18.7%)	Bowles 1999 (352)
Herpes simplex virus	Myocarditis	2/38 (5.3%)	Martin 1994 (344)
Cytomegalovirus	Myocarditis	1/38 (2.6%)	Martin 1994 (344)
	AIDS	3/32 (9.4%)	Bowles 1999 (352)

DCM – dilated cardiomyopathy; PCR – polymerase chain reaction

myocytes not typical of ischemic damage associated with coronary artery disease" (273). However, most patients who were suspected of myocarditis did not meet these criteria. In the Myocarditis Treatment Trial, the utility of the criteria were examined by the pathology panel, which blindly interpreted biopsies. When myocarditis was diffuse, a 92% concordance was noted and, even when focal, concordance was 78% (274). The "Dallas" criteria also defined "borderline" myocarditis as either an inflammatory infiltrate that was too sparse or the lack of myocyte injury. The panel recommended repeat biopsy, and its validity was later confirmed by identification of active myocarditis on repeat biopsy in 67% of those patients whose initial biopsy showed borderline myocarditis (275).

Within the context of inflammatory cardiomyopathy in the new definition by WHO/WHF from 1995 (1), terms such as active/acute or chronic myocarditis, the association between pericardial disease and myocarditis (perimyocarditis), autoreactive or virally induced myocarditis needed further explanation and diagnostic consensus. Therefore, the WHF Council on Cardiomyopathies formed two expert committees, one with international experts on histopathology and immunohistochemistry, and another one with international experts on the molecular diagnoses of infective or viral cardiomyopathies, which convened in separate sessions in Marburg, Germany. Both committees formulated new definitions on chronic myocarditis and inflammatory DCM and on viral cardiomyopathies.

8.3.1. Expert Committee on the Histology of Dilated Inflammatory Cardiomyopathy – DCMI

The committee defined myocarditis as a process characterized by an inflammatory infiltrate of the myocardium. In acute (active) myocarditis, necrosis and/or degeneration of adjacent myocytes are required, whereas in chronic myocarditis, necrosis is not an obligatory feature by definition (Figure 1). When referring to the Dallas criteria, the term acute myocarditis corresponds to active myocarditis; chronic myocarditis may be defined as comprising borderline or healing myocarditis. The inflammatory infiltrate should be sub-classified as lymphocytic, eosinophilic, neutrophilic, giant cell, granulomatous, or mixed. The distribution should be classified as focal, confluent, or diffuse, respectively.

The panel has chosen for the definition of myocarditis a minimum of 14 infiltrating leukocytes/mm², preferably T-lymphocytes (CD45RO) or activated T-cells (e.g. CD45RO) + (up to 4 macrophages may be included in this total amount). The total number is more than two standard deviations above the number of leukocytes found in control tissue (1, 276, 277). In case of nests of leukocytes (≥3 lymphocytes, preferably T-cells) located outside the lumen of a vessel, a focal inflammatory process (myocarditis) is diagnosed. If foci of T-lymphocytes are present, myocarditis can be diagnosed due to the nature of the infiltrate even when the critical number of 14 leukocytes/mm² is not reached. If the focal or diffuse leukocytes are localized in fibrotic areas, the process may be termed reparative.

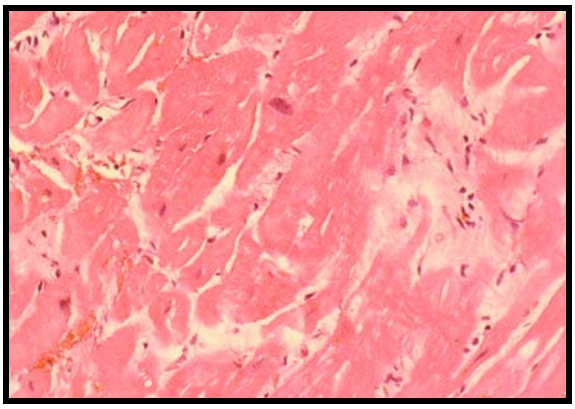

Figure 1. Histology of dilated cardiomyopathy hypertrophied and attenuated myocytes, variation of myofiber size, different degree of fibrosis, and occasional mononuclear cells in the interstitium.

The amount and distribution of fibrosis should be described similarly as no fibrosis (grade 0), mild (grade 1), moderate (grade 2), or severe (grade 3). Localization or formation of fibrosis should be outlined as endocardial, replacement or interstitial. Thus the following terminology was adopted:

8.3.1.1. First biopsy

1. Acute (active) myocarditis: A clear-cut infiltrate (diffuse, focal or confluent) of ≥14 leukocytes/mm² (preferably activated T-cells). The amount of the infiltrate should be quantitated by immunohistochemistry. Necrosis or degeneration are compulsory, fibrosis may be absent or present and should be graded.

2. Chronic myocarditis: An infiltrate of ≥14 leukocytes/mm² (diffuse, focal or confluent, preferably activated T-cells). Quantification should be made by immunohistochemistry. Necrosis or degeneration are usually not evident, fibrosis may be absent or present and should be graded.

3. No myocarditis: No infiltrating cells or <14 leukocytes/mm².

8.3.1.2. Subsequent biopsies

1. Ongoing (persistent) myocarditis. Criteria as in 1 or 2 (features of an acute or chronic myocarditis).

2. Resolving (healing) myocarditis. Criteria as in 1 or 2 but the immunological process is more sparse than in the first biopsy.

3. Resolved (healed) myocarditis. Corresponds to the Dallas classification.

The WHF expert committee on the histology of inflammatory cardiomyopathy introduced chronic myocarditis as a histologically defined independent category (presence of a diffuse or focal leukocytic infiltrate or foci of lymphocytes associated with the presence of myocellular hypertrophy, focal or diffuse interstitial, replacement and/or perivascular fibrosis and non obligatory microvascular changes) for dilated cardiomyopathies. The presence of chronic inflammatory cells (e.g. lymphocytes, monocytes or macrophages) defined by histology and/or immunohistochemistry, in association with the cardiomyopathic changes define chronic myocarditis or **DCM** with **i**nflammation - DCMI.

Chronic myocarditis was defined interchangeably with DCM with inflammation or inflammatory cardiomyopathy - DCMI. Considerable variability in the histological diagnosis of chronic myocarditis can often be resolved by immunostaining, which could be helpful in providing more uniform and quantitative criteria for the diagnosis of myocarditis and DCM and for the present and future treatment trials.

8.3.2. Expert Committee on the Definition of Viral Cardiomyopathy

Since isolation of the virus from swabs or tissue is possible only in the acute phase of infection it is unlikely to succeed with this method in patients with longer lasting diseases or chronic infections. Enteroviruses have therefore been effectively isolated only in pediatric patients. A higher sensitivity was achieved with molecular techniques. It is well documented that molecular techniques e.g. gene amplification (Figure 2) are significantly more sensitive than standard histochemical techniques for the detection of viral proteins. Except for HIV, hepatitis C and CMV serological assessment of antiviral antibodies appeared to be of limited diagnostic value with respect to the actual disease status of the patients and for the critical issue if the viral genome is present in the myocardium.

The WHF expert panel reached a consensus on current diagnostic approaches to viral heart disease by means of an international, multicenter and blinded interlaboratory study. Detection of viral nucleic acid in the myocardium was regarded as indicative of virus infection of the heart. The *PCR* technique was selected for this study because of its rapidity, wide availability, high sensitivity, and specificity. *In situ hybridization*, not carried out in this trial, offers near equivalent sensitivity to PCR, combined with localization of the virus on the cellular level, with the draw back of the lack of rapidity. PCR primers can be specifically designed to amplified any member of a particular virus group. The individual agent group can then be identified by direct sequencing of the PCR-product.

The highest sensitivity and reproducibility for the detection of enteroviral genomes were achieved with frozen tissue (100%) in 5 out of 9 centers. Reverse transcription (RT) - PCR of enterovirus RNA from fixed embedded tissue was less reliable, probably with false negatives and the frequent failure to amplify sequences from the processed mRNA of control house keeping genes. Detection of enterovirus sequences in formalin-fixed samples was less convincing. The incidence of hepatitis C virus in formalin fixed tissue (15%) was remarkable. PCR for the genomic sequences of DNA viruses in formalin fixed tissue is less critical and adeno- (12.5%-22.5%), cytomegalo- (5%) and Epstein-Barr

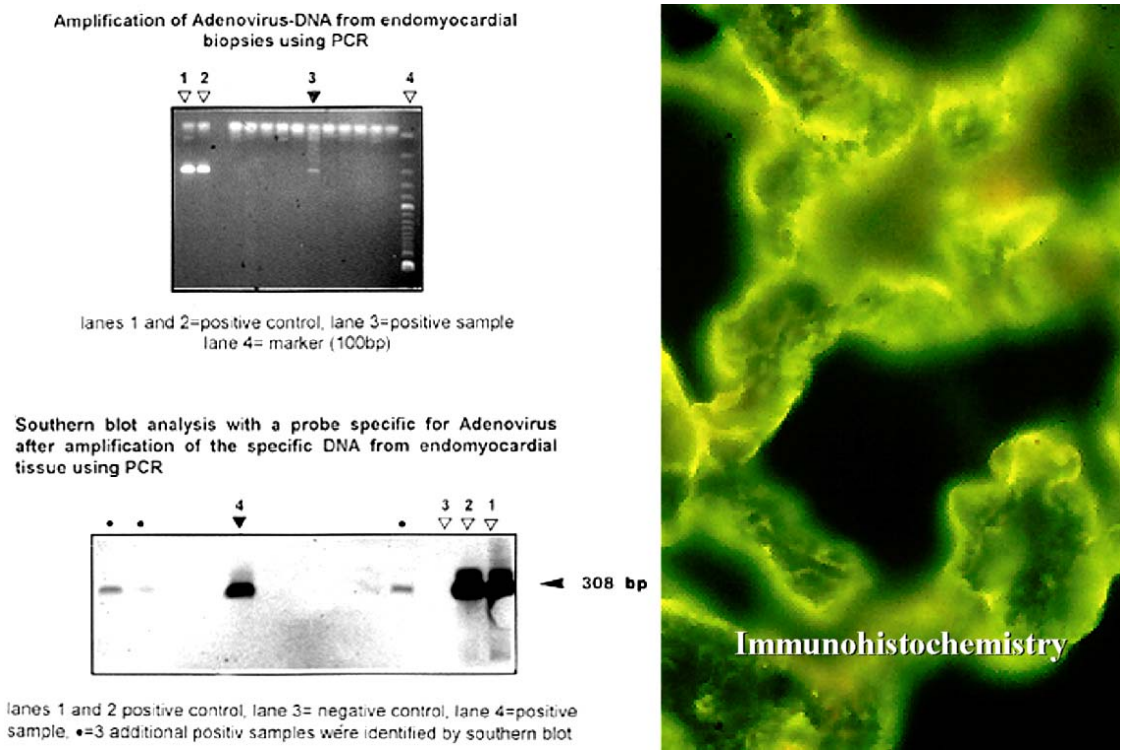

Figure 2. Adenovirus positive dilated cardiomyopathy: PCR (upper left), Southern blot (lower left) and immunohistochemistry findings (right). The PCR demonstrates one positive sample (Lane 3). This finding was confirmed using the Southern blot analysis. The technique is ten times more sensitive than PCR and therefore in addition to the confirmation of the positive PCR findings shown in upper-left image, three additional adenovirus-positive patients were identified using Southern blot analysis.

virus (2.5%) could also be detected in formalin fixed tissue.

As entero- and adeno-viruses are probably the most common agents of viral heart muscle disease, reverse transcription-PCR is required to amplify these viral genomic RNA sequences. The centers' experience was that fresh frozen tissue (biopsy) is the material of choice, giving high sensitivity and specificity of detection. Nested PCR seems desirable to detect a low copy number of enteroviral RNA in chronic disease, but single-step PCR with Southern blot gave equivalent positive results in the tissue samples and dilution experiments in this study.

An advantage of PCR over slot-blot or *in situ* hybridization techniques is that where various members of a virus group may be etiologic, group-specific primers can be used to amplify viral sequences and the particular agent can be identified subsequently by direct nucleotide sequencing of the PCR product.

On the basis of the interlaboratory analyses the second **WHF expert panel on viral cardiomyopathies** has given for the first time a reliable comparative analysis of cardiac tissue samples infected in part with cardiotropic viruses. The high reproducibility of results for enterovirus positive samples in frozen material by the methods outlined here is an important step for the standardization of diagnostic criteria on viral or inflammatory cardiomyopathy. It has also clearly demonstrated that hepatitis C is a RNA virus to be considered, as are DNA viruses e.g. adeno- and cytomegalovirus.

9. MANAGEMENT

9.1 Treatment of Cardiac Dysfunction

Patients presenting with congestive heart failure or asymptomatic left ventricular dysfunction should be treated according to the ACC/AHA Practice Guidelines (278). The role of diuretics (including spirinolactone), digitalis, and angiotensin-converting enzyme inhibitors does not differ in patients with congestive heart failure due to viral cardiomyopathy. However, in the animal myocarditis model, captopril had a beneficial effect (279), while beta-blockade with metoprolol had a deleterious effect (280). In addition, digoxin increased both expression of proinflammatory cytokines and mortality in the murine model of viral myocarditis and should be used with caution and only at low doses (281). Aggressive therapy to lower vascular filling pressures might minimize immune activation by preventing the release of endotoxin in the gut (282).

Although recommendations regarding physical activity, and even exercise training, are evolving in patients with congestive heart failure, the diagnosis of myocarditis traditionally called for restriction of physical activity. Experimentation with Coxsackie myocarditis in animal

models demonstrated that exercise enhances mortality and myocardial injury, augmenting the intensity of infiltration by cytotoxic T lymphocytes and necrosis (283, 284). Exercised animals also show enhanced cardiac dilatation (285). Although no comparable studies have been performed in men, these theoretical deleterious effects have led to recommendations for exercise restriction. If mural thrombosis is found, systemic anticoagulation is warranted (286).

Ventricular arrhythmia is common in patients with active viral infection, and in most cases does not require specific therapy. However, occasional patients will present with severe refractory ventricular arrhythmia, and consideration for antiarrhythmic therapy should be given. Because spontaneous remission may occur, a commitment to long-term antiarrhythmic therapy—such as amiodarone or implantable cardioverter/defibrillators (ICD)—should only be given after all means of controlling ventricular arrhythmia have been unsuccessful. Patients with atrioventricular block may require insertion of a temporary pacemaker. However, atrioventricular block is typically transient and insertion of a permanent pacemaker is only rarely indicated.

When congestive heart failure due to other etiologies is progressive and refractory to conventional therapy, cardiac transplantation is commonly considered (287). It was assumed that the outcome of cardiac transplantation in patients with active myocarditis would parallel the excellent results in ischemic cardiomyopathy. However, retrospective analysis utilizing the data base of the International Society for Heart and Lung Transplantation demonstrated that patients with active myocarditis had severe, frequent, early rejection with a higher mortality (288). As an explanation for this observation, the immunologic milieu of activated lymphocytes and antibodies directed toward cardiac antigens may theoretically enhance the immune reactivity. When recipients with serum anticardiac antibodies prior to transplantation were compared to those without, the former had more severe rejection and twice the rejection frequency (289). In addition to the predisposition to early severe cardiac allograft rejection, inflammatory heart disease may recur in the allograft. The literature has multiple case reports of recurrence of giant cell myocarditis following cardiac transplantation (290-293). The confirmation of recurrence of active lymphocytic myocarditis is very difficult, since the histologic appearance resembles cardiac allograft rejection. Although it is highly suspicious that myocarditis recurs, likely it is misdiagnosed as cardiac allograft rejection.

Intense medical therapy and mechanical circulatory support should be given before consideration for transplantation. This observation period allows sufficient time for spontaneous improvement. Multiple reports of patients who required mechanical circulatory assistance for weeks and spontaneously recovered to totally normal cardiac function have been particularly encouraging (294-296). The combination of an adverse outcome following cardiac transplantation and the possibility of recurrence in the allograft coupled to a high spontaneous improvement rate of the disease make the patient with active viral infection a poor candidate for cardiac transplantation.

9.2. Antiviral and Immunosuppressive Therapy

Intense interest in immunosuppression for inflammatory heart disease followed the initial report from 1953 of a seven-year-old with a viral syndrome and severe cardiac failure who dramatically improved after initiation of treatment with ACTH (297). Despite the limitations imposed by the lack of tissue diagnosis, early clinical studies reported optimistic results of immunosuppressive therapy, particularly in children with clinically diagnosed acute myocarditis (298-300). When patients with new onset heart failure and diffuse myocardial gallium-67 uptake were treated with prednisone and azathioprine, 40% showed improvement by left ventricular ejection fraction and reduced mortality (301). However, in two prospective randomized clinical trials of prednisone in DCM, no long-term benefit could be detected (230, 302).

In the initial report of biopsy-proven myocarditis in patients with unexplained congestive heart failure, the histopathology was reminiscent of cardiac allograft rejection and immunosuppressive therapy (rejection treatment) was attempted (208). Following administration of prednisone and azathioprine, 50% of the treated patients improved. This report stimulated a flurry of mostly optimistic results of immunosuppressive therapy (37, 216,223, 230, 231, 303-309). Similarly, when patients with refractory ventricular arrhythmia and biopsy-proven myocarditis were treated with immunosuppression, results were even more favorable (245, 247, 248, 310). Even more aggressive immunosuppression with the monoclonal antibody OKT3 was favorable in isolated case reports (311, 312). Removal of antibodies by immunoabsorption was advantageous in patients with heart failure and high levels of anticardiac antibodies (313, 314). In peripartum myocarditis 90% of patients improved, prompting the investigators to recommend immunosuppression for all patients with peripartum cardiomyopathy and histologic evidence of myocarditis (262).

Some investigators are approaching documented viral persistence aggressively with antiviral therapy or immunoaugmentation with interferon to enhance viral clearance. Although the antiviral agent ribavirin had no beneficial effect (315), interferon alpha resulted in improvement in 73% of patients in a preliminary clinical trial (316), which was not randomized, thus leaving open the question of spontaneous improvement. In addition, intravenous immune globulin caused marked improvements in left ventricular performance in children (176) and adults (317) with recent onset of symptoms of heart failure and in women with postpartum cardiomyopathy (318). However, histologic resolution of myocardial inflammation does not always necessarily correlate with improvement in ventricular function.

The clinical value of antiviral therapy is being assessed in the European Study of Epidemiology and

Table 5. European Study of Epidemiology and Treatment of Cardiac Inflammatory Diseases (ESETCID) - study protocol (319, 320)

Design of the study:
multi-center, prospective, randomized, placebo controlled, double blind study in patients with biopsy proven active or chron myocarditis, or dilated cardiomyopathy with inflammation.
Inclusion criteria:
Initial diagnosis of active and/or healing myocarditis according to the Dallas criteria or at least 14 activated lymphocytes macrophages/mm² detected by immunohistochemistry in the endomyocardial biopsy, and a left ventricular ejection fracti (LVEF) ≤ 45%.
Treatment arms:
1. CMV myocarditis and CMV-persistence Hyperimmunoglobulin: 1 time per day 4ml/kg BW on day 0, 4 and 8 2ml/kg BW on day 12 and 16 vs. placebo
2. Coxsackie B myocarditis Interferon alpha: 2,5 Mio. IU/m² surface area s.c. 3 x per week vs. placebo
3. Adenovirus or Parvo virus B19 -positive myocarditis Immunoglobulin treatment: 10g intravenously at day 1 and 3 vs. placebo
4. Autoimmune myocarditis and perimyocarditis (CMV, ADV, PVB 19 and enterovirus negative) Prednisolone: (1.25mg/kg/day for 4 weeks, maintenance dose: 0.3 mg/kg/day) + Azathioprine (2 mg/kg/day for 2 weeks, maintenance dose: 0,85 mg/kg/day) vs. placebo
The primary endpoints:
1. Improvement of ejection fraction (by radionuclide-scintigraphy) by more than 5%
2. Improvement of exercise tolerance (bicycle ergometry) by >10% of the previous exercise capacity
Secondary endpoints:
1. Reduction of left ventricular enddiastolic volume index
2. Ejection fraction in gated blood pool scintigraphy during exercise
3. Resolution of the inflammatory infiltrate
4. Improvement of life-style as assessed by questions regarding quality of life
5. Elimination of viral DNA or RNA

Treatment of Cardiac Inflammatory Diseases (ESETCID) in our center (319, 320). In the ESETCID study, patients with LVEF≤45% and PCR positive for enterovirus are randomly assigned to treatment with interferon alpha or placebo, adenovirus-positive patients are randomized to immunoglobulins and placebo, and cytomegalovirus-positive patients are randomized to hyperimmunoglobulins and placebo (Table 5).

Several randomized controlled studies have been conducted for evaluating an anti-inflammatory regimen in DCM and/or myocarditis. The Myocarditis Treatment Trial was designed to assess the role of immunosuppressive therapy in myocarditis (321, 322). Patients with active myocarditis by the "Dallas" criteria were randomized to receive immunosuppressive therapy with prednisone and azathioprine, prednisone and cyclosporine, or no immunosuppression for six months. The primary endpoint for the clinical trial was ejection fraction twelve months after randomization. Endomyocardial biopsy was performed on 2233 patients, 214 had active myocarditis, and 111 were randomized. When subjects receiving immunosuppression were compared to controls, no difference in cumulative mortality was detected. A comparable rise in ejection fraction of approximately 0.10 in both groups confirmed the high likelihood of spontaneous improvement in patients with active myocarditis. Therefore, no effect of immunosuppression could be demonstrated.

Parillo *et al.* (302) studied 102 patients with DCM who had evidence of inflammation (n=60, reactive patients) or no evidence of inflammation (n=42, non-reactive patients). Each group was given conventional heart failure medications. Reactive and non-reactive groups were separately randomized to prednisone or placebo. At 3 months, 67% of reactive patients who received prednisone had improved LVEF by ≥ 5% vs. 28% of the reactive control group (p=0.004). The nonreactive patients did not improve with prednisone. At 3 months, the patient regimen was changed from daily prednisone to alternate day prednisone and on this regimen, initial LVEF improvement was no longer seen at 9 months from the study initiation. No difference was seen in mortality between the control and immunosuppressive group.

Another controlled trial randomized 62 DCM patients to therapy with immunoglobulin or placebo (317, 323). No treatment effect was seen, although both groups had a 14% increase in LVEF. The study lacked biopsy confirmation of the underlying disease, however.

Recently, Wojnicz *et al.* (324) used immunohistochemistry on endomyocardial biopsy specimens to identify upregulated HLA expression in 202 patients with DCM. HLA-positive patients (84/202) were randomized to immunosupression with prednisone and azathioprine or placebo for 3 months. After a follow-up of three years, the primary end-point (a composite of death, heart transplantation, or hospital readmission) did not differ between the study groups. However, the immunosupressed patients demonstrated significant increase in LVEF both at 3 months and 2 years. Similar improvements were also evident at 2 years in NYHA functional class. In contrast to the previous studies, Wojnicz *et al.* have included only patients with chronic congestive heart failure, which could

represent the more appropriate group for immunosuppressive therapy. In this way, the large proportion of spontaneous improvement of acute heart failure in the control group was avoided. This study did not however, report on the analyses of viral or bacterial DNA or RNA in the biopsy specimens, thus leaving the question of etiology unanswered.

10. SUMMARY AND PERSPECTIVE

Viral infection of the heart is relatively common and usually asymptomatic and with spontaneous and complete resolution. It can, however, lead to substantial cardiac damage, development of viral cardiomyopathy and severe congestive heart failure.

Humoral autoimmunity in postviral heart disease remains to be an attractive but controversial hypothesis addressing the pathophysiology of human viral cardiomyopathy. Antigenic mimicry with or without cytolytic antibody properties has been shown to play a role in the immunopathogenesis of myocarditis with respect to sarcolemmal/ myolemmal epitopes (including the beta-receptor), myosin and some mitochondrial proteins, including ANT-carrier and dihydrolipoamid dehydrogenase. The measurement of cardiac antibodies has great research interest, but little clinical application, and does not justify immunosuppression.

The diagnosis of myocarditis and viral cardiomyopathy can be made only by endomyocardial biopsy, implementing the WHO/WHF criteria and PCR techniques for identification of a viral genome. Several studies have lent convincing support to the hypothesis that low level expression of viral genome can induce chronic dynamic myocardial injury. Patients with active myocarditis who deteriorate despite maximum medical management may be considered for immunosuppression. If improvement occurs, the physician must be aware that the improvement may be spontaneous and unrelated to immunosuppression. However, there is no evidence that immunosuppression is harmful. The threshold for anticoagulation should be low, and exercise restriction should be imposed. Cardiac transplantation should only be considered when all other options, including mechanical circulatory assistance, have failed and no contraindications exist. The hemodynamic support of patients with acute left ventricular failure caused by viral cardiomyopathy should be aggressive, to allow for the possibility of spontaneous recovery. Ongoing evaluation of antiviral therapies, removal of antibodies by immunoabsorption, virus-specific vaccines, and mechanical support devices may provide new treatment options. The recent recognition of the genetic predisposition for the viral infection of the heart should also provide valuable clues to the understanding and treatment of this challenging disease.

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Abbreviation: WHO: World Health Organization

Note: The expert committee on histopathology and histochemistry included B. Bültman, S. Factor, H-J Gröne, G. Hufnagel, K. Kawamura, U. Kühl, B. Maisch, E.J. Olsen, S. Pankuweit, R.Virmani, Invited consultants were W. McKenna, P.J. Richardson, G. Thiene, H-Peter Schultheiß, M. Sekiguchi. The expert committee on viral heart disease included Ch. Aepinus, K. Aitken, E. Arbustini, L. Archard, C. Baboonian, N. Bowles, S. Broor, G. Hufnagel, R. Kandolf, P. Liu, B. Maisch, A. Matsumori, W. McKenna, S. Pankuweit, M. Pauschinger, H-P. Schultheiß, W. Slenczka, M. Sole, K.K. Talwar, J. Towbin, S. Tracy. Invited consultants were: A. Bayes de Luna, J.F. Goodwin, P.J. Richardson.

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