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Myocarditis—Personalized Medicine by Expanded Endomyocardial Biopsy Diagnostics

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Abstract

Myocarditis and dilated cardiomyopathy (DCM) are acute or chronic disorders of myocardium. The gold standard for final confirmation of causative reasons of these heart muscle diseases is the endomyocardial biopsy (EMB) analysis. Due to focal pathology, diagnostics are failing if the EMB does not contain the area of interest. Personalized medicine comprises the genetic information together with the phenotypic and environmental factors to yield a tailored healthcare for each individual and removes the limitations of the "one-size-fits-all" therapy approach. This provides the opportunity to translate therapies from bench to bedside, to diagnose and predict disease, and to improve patient-tailored treatments based on the unique signatures of a patient's disease. Furthermore, novel treatment schedules can be identified which have eventually the chance to enhance long-term survivals. Global biomarkers such as specific gene expression signatures or miRNA profiles not only have the potential to reduce this problem but also add valuable information for individualized therapy decisions. In future, multiplex approaches allowing rapid and absolutely reliable identification of inflammatory or virally-induced myocardial diseases will replace singleplex methods such as direct detection of viral genomes in one single biopsy. Gene or miRNA profiles are upcoming diagnostic biomarkers for cardiomyopathies which are not only detectable in tissue samples but in body fluids as well. Consequently, a systemic diagnostic approach by determination of distinct expression pattern in e.g., peripheral blood samples will support the characterization of distinct cardiomyopathies by means of non-invasive methods.

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Keywords

Cardiomyopathy, Endomyocardial Biopsy, Personalized Medicine, Biomarker, MicroRNAs

1. Introduction

Cardiovascular disease is a leading cause for both hospitalization and death in Western European Countries. Its prevalence is rather increasing with the broad implementation of standardized evidence-based treatment algorithms for heart failure [1]-[4].

Myocarditis and dilated cardiomyopathy (DCM) are acute or chronic disorders of myocardium. According to surveys of the European Society of Cardiology (ESC), 12 million European patients are suffering from heart failure including two million with non-ischemic dilated cardiomyopathy. DCM is the leading cause of heart transplantation (45%) of heart failure patients below 60 years of age in the US. The incidence of DCM is 5 to 8 per 100.000 and the prevalence is about 36 per 100.000 in United States [5]. These data are based on post-mortem studies regardless of the more frequent findings of DCM in endomyocardial biopsies (EMB). Based on this assumption, the worldwide incidence of DCM is at least above 50 million patients and thereby higher as the official number for human immune deficiency virus (HIV) (35 Mill. patients worldwide) as a well-known global health problem (UNAIDS Report 2012).

The individual patient's clinical symptoms, medical and family history, and data from laboratory and imaging evaluations are in the focus of traditional clinical diagnosis to confirm and treat illnesses.

Improvements in human genetics have enabled a more detailed understanding of the impact of genetics in disease. Large collaborative research projects e.g., the Human Genome Project brought forward the understanding of the roles of genes in normal human development and physiology, revealed single nucleotide polymorphisms (SNPs) that account for some of the genetic variability between individuals, and made the use of genome-wide association studies possible to examine genetic variation and risk for many common diseases [6] [7]. In consideration of the continuously expanding field of biomarker discovery in terms of proteomics, metabolomics and transcriptomics, physiological biomarkers like miRNA or gene expression profiles are belonging to personalized medicine as well, in our opinion.

Personalized medicine is a kind of medicine that uses the patient's individual genomic information to improve diagnosis, prevention and therapy. In this review, we discuss the personalized management of myocarditis and DCM as monogenic or multifactorial disorders which require multistep diagnostics for an optimized treatment decision [8].

2. Acute Myocarditis

The term myocarditis describes inflammatory disorders of the heart muscle of varied infectious and non-infectious origin. It can be caused by any kind of infection, drugs, toxic substances, or be associated with autoimmune conditions. Infectious etiologies include a vast number of viruses, bacteria, protozoa or fungi, but most frequently the myocardial inflammatory process is directed against viral pathogens. Viruses are the main cause in industrialized countries while bacteria or other infectious agents predominate the etiology of myocarditis in developing countries [9]. In common, the histological patterns of myocarditis are categorized by the predominant inflammatory cells and can be divided into lymphocytic (including viral and autoimmune forms), neutrophilic (bacterial, fungal, and early forms of viral myocarditis), eosinophilic (hypersensitivity myocarditis or hypereosinophilic syndrome), and granulomatous (cardiac sarcoidosis and giant cell myocarditis). The actual incidence of virus induced myocarditis or virus-associated cardiomyopathy is not well established because viral heart diseases can be unapparent, are difficult to diagnose and can vary with different viruses as a function of circulating virus populations (**Figure 1**).

Outcome and prognosis of myocarditis depend on etiology, clinical presentation, and disease stage. In acute phase, the clinical presentation can range from asymptomatic ECG alterations with or without echocardiographic abnormalities, to infarct-like presentation, global cardiac dysfunction, arrhythmias or progressive heart failure and cardiac decompensation [1]-[4].

In contrast, the symptoms in subacute myocarditis and inflammatory cardiomyopathy are more subtle and

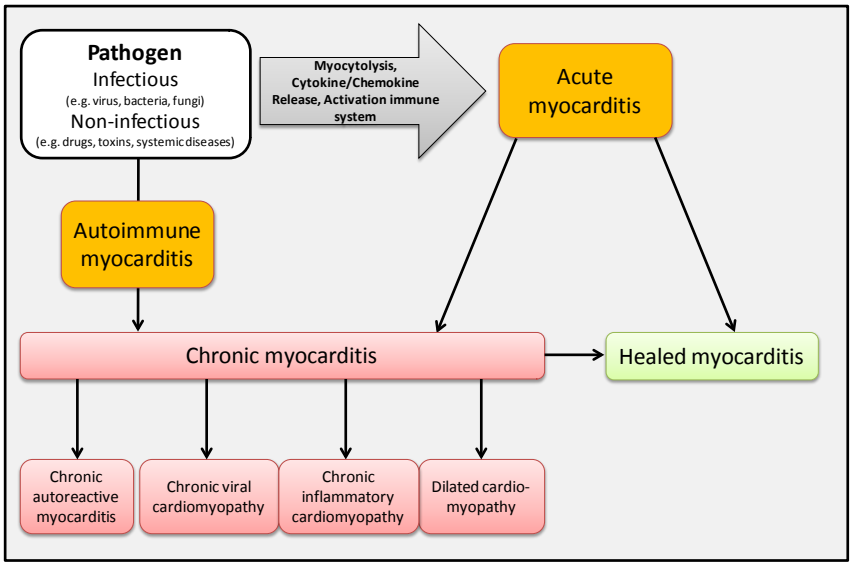

Figure 1. Pathogenesis and evolution of acute myocarditis to dilated cardiomyopathy (adapted from [2]).

insidious, often presenting as dilated cardiomyopathy with fatigue, reduced physical capacity and dyspnea. Under specific circumstances acquired diseases are indistinguishable from hypertrophic (HCM), restrictive (RCM) or arrhythmic right ventricular cardiomyopathy (ARVC), clinically.

In about 50% of cases, acute myocarditis resolves in the first 4 - 12 weeks, but approx. 25% will develop persistent cardiac dysfunction and 12% - 25% may acutely deteriorate and either die or progress to end-stage DCM with a need for heart transplantation [1] [2]. Biventricular dysfunction at presentation has been reported as the main predictor of death or transplantation. Fulminant myocarditis is said to differ from (sub)acute lymphocytic myocarditis in its mode of onset, degree of hemodynamic compromise, and better outcome, but data are relatively scarce in adult patients.

Idiopathic giant cell myocarditis (IGCM), acute eosinophilic myocarditis and granulomatous inflammatory processes such as cardiac sarcoidosis (CS) are rare but clinically important acute inflammatory heart muscle diseases of often unknown etiology [10]. The majority of patients present with acute heart failure upon first encounter. These myocarditis subtypes have a high mortality if not diagnosed and treated in time. Drugs may induce myocardial inflammation by either direct toxic effects on heart tissue or by inducing hypersensitivity reactions which are often associated with an eosinophilic myocarditis [11]. Eosinophils are also observed in myocardial inflammatory processes which are associated with Churg-Strauss vasculitis or hypereosinophilic syndromes, vaccination for several diseases or caused by helminthic and parasitic infections [1] [2]. Chronic myocardial inflammation may be caused by persisting organ infections, toxic agents or distinct physical conditions, persist as a post-infectious condition, or be associated with systemic autoimmune conditions.

Because of the non-specific symptoms and clinically indistinguishable etiological factors the prevalence of infectious and non-infectious causes of myocarditis is not known. Biopsy of the myocardium is the only way to reach a treatment relevant diagnosis [2] [12]-[14].

3. Pathogenesis of Myocarditis and Inflammatory DCM

Viral infections of the heart are progressing within distinct pathologically phases. A direct virus-related cytolysis of cardiomyocytes is already detected before any inflammatory infiltrate develops and appears to be decisive in fulminant cases of myocarditis [14]-[18]. Resulting myocyte necrosis may cause a significant loss of contractile tissue which is accompanied by rapidly developing cardiac failure and early death of the host. Early antiviral defence mechanisms of the innate immune system additionally contribute to early myocardial lesions and impaired myocardial function.

The activation of antigen-specific cell mediated immunity initiates the second phase of virus clearance. Virus-infected cells are destroyed by immune effector cells of the emerging acquired inflammatory response;

therefore virus clearance will occur at the expense of further loss of infected myocytes. The ensuing myocardial damage depends on the scale of the cellular virus infection and increases with growing virus dispersion which, in addition to the early virus- and immune-mediated injury, contributes to tissue remodelling and possible progression of the disease (**Figure 1**). The healing process therefore results primarily from a partial destruction of myocardial tissue that is not capable of regeneration. This finally may account for a clinical picture that is consistent with an often irreversible dilated cardiomyopathy. When myocardial inflammation persists despite virus clearance, a chronic myocarditis or inflammatory cardiomyopathy is present (**Figure 1**). It is generally accepted that viral myocarditis plays a major role in the development of inflammatory cardiomyopathy [2] [13] [19].

In the third remodelling phase, virus infection may have been cleared completely and antiviral immune responses may have been resolved regularly. The extent of the myocardial damage determines the further clinical course of these patients whose biopsy results at that time will be that of idiopathic DCM. A post-infectious disease (healed myocarditis and DCM) can only be diagnosed if history or previous diagnostics had proven a preceding infectious or inflammatory state. On the other hand, latent virus infection, virus-associated low-grade immune reactivity and/or autoimmune processes may continuously assert negative effects on myocardial performance. Biopsy would then be consistent with inflammatory cardiomyopathy or persisting viral heart disease. Without sophisticated diagnostic analyses, the clinical picture is indistinguishable from that of DCM.

Long-term follow-up studies of patients presenting an acute myocarditis have shown that approximately 21% of them develop DCM. Additionally, the presence of a viral genome was demonstrated by polymerase chain reaction in the myocardium in up to 67% of patients with idiopathic left ventricular dysfunction [15] [20]. Thus, DCM can occur as a late stage following cardiac infection and inflammation. In contrast to acute myocarditis, which often presents with a preserved left ventricular size and normal or even increased wall thickness due to edema, inflammatory cardiomyopathy is characterized by the presence of chronic inflammatory cells associated with left ventricular dilatation, wall thinning and reduced ejection fraction, with or without viral persistence [16] [19]. Different mechanisms have been suggested for this evolution from acute disease to DCM [15] [21]-[23].

4. Infectious Causes of DCM

The major causes of myocarditis and inflammatory cardiomyopathy (DCMI) in acquired “idiopathic” diseases of the heart muscle [9] [24] are infectious agents and while practically any microbial agent can cause myocardial inflammation and dysfunction, non-viral infections are rare in these conditions, at least in developed countries. Nowadays viral forms are considered to be the most common cause of acquired DCMI [19] [24].

In pediatric as well as in adult myocarditis and chronic heart muscle disease, coxsackieviruses [14] [17] and, to a lesser extend adenoviruses [17] [25] , are well established. Additionally, with varying degrees of frequency distinct genotypes of erythroviruses including parvovirus B19 (B19V), human herpesvirus type 6 (HHV6A/B) and its chromosomally integrated form (ciHHV6 A/B), HIV, cytomegalovirus (CMV), herpes simplex type 2 virus, Epstein-Barr virus (EBV) and hepatitis C virus, among many others, have been identified in cardiac tissues [26]-[29].

Using molecular biological techniques including nested polymerase chain reaction (nPCR), viral genomes have been identified in myocardial tissues of approximately 30% to 73% of patients who underwent EMB in order to clarify the etiology of heart failure. Some more recent studies from Western Europe and the US have demonstrated a decrease in the prevalence of enteroviruses and adenoviruses as pathogens while erythroviral and herpesviral genomes became detectable in higher frequencies with erythroviruses B19V as the most frequent viruses detected in EMBs [15] [18] [25] [30] [31]. Concerning the higher frequencies of erythrovirus and herpesvirus, it has to be kept in mind that these viruses lead to a lifelong persistence after childhood infection [32] [33]. Therefore this detection in different tissues particularly in adults often represents latent infections. Virus-associated symptomatic diseases are normally caused by reactivation of persisting pathogens [1] [24]. Persistent latent infection of B19V occurs in the vascular endothelium of different organs including the heart with localisation of the virus in endothelial cells of venuoles, small arteries or arterioles of patients with fulminant myocarditis or sudden onset heart failure [34]-[36]. Reactivated erythrovirus is often associated with symptomatic endothelial dysfunction whereas latent infection is, in the majority of cases, asymptomatic [37].

Another widespread latent virus infection frequently detected in EMBs is HHV6 [15] which consists of two genetically related but biologically distinct groups—HHV6A and HHV6B. After primary infection in childhood the virus persists in more than 70% of the adult populations [38]. In addition to lymphocytes, HHV6 also infects a variety of other cell types including cardiomyocytes and endothelial cells [39] [40]. Another interesting fact is

that HHV6 can integrate into the telomeres of human chromosomes. ciHHV6 affects 0.4% - 0.8% of the whole population and is transmitted via the germline [33] [41]. Chromosomal integration with permanent virus reactivation may cause rarely recognized symptomatic diseases including cardiomyopathies. HHV6 and ciHHV6 become frequently reactivated with subacute clinical presentations.

Given the fact that a qualitative similar spectrum of viruses is detected in EMB of patients with acute and chronic myocarditis as well as in EMB of patients with DCM/DCMI, these diseases are considered to be different stages of one acquired disease rather than different disease entities [5] [15]. Virus persistence is associated with a progression of heart failure whereas patients improve clinically when viruses are cleared spontaneously or upon antiviral treatment [20].

5. Diagnosis and Prognosis of Myocarditis and Inflammatory Cardiomyopathy

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 [16] [19] [20] [26] [42]. Cardiac involvement is often considered as the differential diagnosis only when cardiac symptoms, such as palpitations, angina, and/or exertional dyspnea, persist for a long period after the initial infection has resolved, or if they develop *de novo* in the course of the recovery. At this point in time, the electrocardiography results and laboratory chemical findings that are characteristic of acute myocarditis (changes of the ST segment, raised cardiac enzymes that are typical for acute myocardial involvement) are no longer present. Diagnostic evaluation starting at this point can only collect data on the extent of myocardial injury, even when using imaging methods (echocardiography, angiography, magnetic resonance imaging [MRI]); exclude specific cardiac disorders such as ischemic cardiomyopathy or valve deficiencies; or provide clues for a suspected diagnosis of infectious myocarditis. However, it cannot diagnose the cause of the existing disorder. While 60% to 70% of patients improve clinically and hemodynamically, the remaining patients will develop chronic heart failure or dilated cardiomyopathy within months or years [22] [43].

An unequivocally confirmed bioptic diagnosis is the crucial prerequisite for differential diagnostic evaluation and the specific treatment strategies derived from this. As the pathophysiological changes of infectious and non-infectious myocarditis occur at a cellular and subcellular level, confirmation of a specific pathogen or inflammation requires a direct examination of myocardial tissue, which can be obtained without problems by means of a biopsy [26] [44]. In terms of therapeutic considerations it needs to be taken into account that a positive treatment effect will occur only if treatable causes such as viral infections, inflammatory processes, or cardiodepressive autoantibodies are present and the myocardium still has regenerative potential [4]. If irreversible myocardial damage already occurred, for which no specific treatment options exists such as in postinfectious or postinflammatory dilated cardiomyopathy, the development or progression of heart failure in the long term cannot be prevented.

Acute myocarditis mostly does not sufficiently respond to symptomatic medication for heart failure and mortality is still high despite treatment. The long-term disease course depends on the pathogen, the extent and type of inflammation, and the degree of injury to the myocardium. Focal borderline myocarditis often undergoes spontaneous clinical healing if no serious heart failure has developed initially. The early mortality of fulminant lymphocytic myocarditis requiring intensive care is greater than 40% in the first month [45]. Untreated IGCM and eosinophilic myocarditis also have an extremely poor prognosis, with a 4 year survival rate of 11% [10]. Granulomatous necrotizing myocarditis is fatal if overlooked and untreated. Non-fulminant active myocarditis has a mortality rate of 25% to 56% within 3 to 10 years, owing to progressive heart failure and sudden cardiac death, especially if symptomatic heart failure manifests early on [46]-[48]. In addition to impaired left ventricular (LV) and right ventricular (RV) function, virus persistence, chronic inflammation, and cardiodepressive autoantibodies are independent predictors of a poor prognosis [46] [49] [50].

Damage of cardiomyocytes is not only caused by cytopathic effects of the virus but also by the attracted immune effector cells. Adenovirus and enterovirus are infecting myocytes. Even under optimal heart failure treatment, progression of myocardial injury cannot be prevented [14] [17] [21]. Myocardial function is affected by virus associated inflammation in case of vasculotropic viruses, e.g., erythrovirus or herpesvirus. Infarct like symptoms in these diseases are often induced by injury of the vascular endothelium [28] [34] [51] [52].

Post-infectious inflammation affects myocardial tissue if the inflammatory process does not resolve after successful elimination of the infectious agent [12] [50] [53] [54]. Inflammation in association with collagenosis,

rheumatic disorders or CS is less frequent than virus associated or postinfectious inflammation. Immunosuppression can significantly improve outcome of these diseases and the failing in recognition is fatal in untreated cases. Myocardial damages can be induced by toxic substances or drugs if treatment is not stopped immediately.

If the primary causes of acquired cardiomyopathies, e.g., inflammation or infection have resolved, the etiology of the disease remains unclear (idiopathic) because the causing agents are no longer detectable. In these cases, a specific treatment is impossible. These cardiomyopathy stages cannot be discriminated from other idiopathic or genetic cardiomyopathies [1]-[3].

6. Endomyocardial Biopsy Diagnostics

Since pathophysiological changes of acquired infectious and non-infectious heart muscle diseases, that can be treated specifically by anti-viral or anti-inflammatory medication, occur at cellular and subcellular levels, imaging techniques such as CMR or echocardiography can only provide non-invasive tissue characterization but fail in revealing the true underlying causes that determine prognosis of the disease [55]-[57].

Invasive removal of a sufficient number of tissue samples by EMB is always necessary when an exact diagnosis cannot be obtained by other clinical methods and is influencing the following treatment [26] [27] [44] [58].

EMB is mandatory in suspected myocarditis and DCM of unknown etiology since the diagnosis of myocarditis and other inflammatory diseases (inflammatory cardiomyopathy, cardiac sarcoidosis, giant cell myocarditis), viruses or storage diseases (amyloidosis) is impossible without their direct proof in myocardial tissue [1] [2] [4] [25] [49] [58]. The use of LV EMB to investigate cardiomyopathies is currently discouraged because it is considered riskier than and as contributive as RV biopsy. On the other hand, a recently published study on 4221 patients which underwent diagnostic EMB during a 28-year period confirmed however that even biventricular EMB has a low major complication rate (perforation with or without cardiac tamponade, embolization) of 0.33% for LV EMB and 0.45% for RV EMB. No patient died [27]. These data were in line with a formerly published study (no patient died, major complication rate 0.12%) in which 3048 diagnostic procedures (RV EMB) were taken during an 11-year period [44]. According to these studies, LV or RV EMB is a safe procedure with very low transient complications.

The histological examination of paraffin sections by different staining protocols (HE, EvG, PAS, Azan) detects myocardial cell death, scars, fibrosis, disarrays, cardiomyocyte changes, pathological vascular conditions, granulomas, and inflammatory cell differentiation. Storage disorders such as amyloidosis [26] [59], iron deposits, glycogen and others can be excluded or specified by additional staining, e.g., immunohistochemical differentiation of amyloid subtypes and optional electron microscopical analyses. The EMB diagnosis of myocarditis was based on histomorphological criteria according to the Dallas classification [26] [60].

Routine immunohistochemical diagnostics are based on the application of antibody-supported detection of inflammatory infiltrates (lymphocytes, macrophages, memory cells, B-cells) and enhanced expression of tissue activation marker like adhesion molecules (HLA, ICAM, VCAM) or fibrotic proteins like collagens on cryofixed tissue section, following quantitative digital imaging analysis [26] [61] [62].

Microbial genomes are determined, quantified and sequenced using PCR-based methods. The use of these sensitive techniques detecting the most common cardiotropic viruses (such as enteroviruses, adenoviruses, erythrovirus, human herpesvirus 6, Epstein-Barr virus and in the Far East also hepatitis C) reveals a virus infection in up to 73% of patients who are biopsied under the clinical suspicion of myocarditis or DCM. The clinical significance is clearly demonstrated only for some cardiotropic viruses [2] [15] [17] [20] [25] [51].

7. Current Biopsy-Based Treatment Decisions

Despite expanding knowledge based on molecular and cellular pathophysiology and management of cardiomyopathy, it still remains difficult to accurately distinguish between patients who will someday develop progression of the disease requiring cardiac transplantation from those with excellent long-term prognosis. Of equal importance, current medical practice does not include strategies to tailor therapies to patient's most favorable benefit, while at the same time seeking predictors of poor or adverse responsiveness [63].

During the last decade, the therapeutic management of patients with left ventricular systolic dysfunction has improved by administration of an optimal symptomatic heart failure medication. The major goals of the treatment are the reduction of mortality and morbidity, the improvement of symptoms, and if possible the induction

of reverse remodeling of the left ventricle.

The mainstay of treatment for myocarditis presenting as DCM is an optimal heart failure medical regimen according to the current American Heart Association/American College of Cardiology Foundation and ESC guidelines for the management of heart failure [3] [10] [19].

Virus-negative chronic inflammation (post-infectious or auto-immune inflammatory processes) responds well to immunosuppression or immunoadsorption [5]. If an immunosuppressive treatment is administered in time before irreversible myocardial damages have developed, progression of heart failure can be avoided [49] [58]. Since heart failure of virus positive patients deteriorates upon anti-inflammatory treatment, it is of utmost importance that viral infections are excluded before any immunosuppression is administered.

For antiviral treatment currently two approaches exist: immunomodulation supporting intrinsic immune response and antiviral drugs that affect the life cycle of corresponding virus. Patients with chronic heart failure due to persisting enterovirus and adenovirus infections of the myocardium respond well to a six months' interferon-beta (IFN-β) treatment, whereas untreated patients have a poor prognosis with a mortality rate above 50% [64] [65]. Virus clearance is paralleled by a hemodynamic improvement and an amelioration of heart failure symptoms. The long-term mortality of patients is significantly reduced by the treatment [64].

Other viruses, e.g., B19V or HHV-6, respond less well upon IFN-β treatment with respect to virus clearance. Despite incomplete virus clearance, these patients improve clinically in association with reduction of virus load due to improvement of endothelial function [52].

In 15% - 20% of erythrovirus positive patients, an active virus replication is detectable by measurement of viral RNA or specific miRNA pattern [37] [66]. Transcriptionally active erythrovirus is associated with higher rate of angina, fatigue syndrome or dyspnea. Clinical symptoms of these patients often improve shortly after antiviral treatment with virus replication inhibitors. Whether this also improves outcome is currently unknown.

HHV-6 is the second frequent virus and detectable in over 14% of all EMBs with low viral loads. Its clinical relevance with respect to heart failure is still uncertain [15] [20]. In about 1% of all EMBs, HHV6 could be detected with persistent high viral loads (over 50.000 copies per µg myocardial DNA) characteristic for ciHHV-6. Cardiac complaints and general physical disability improve upon antiviral medication that reduces HHV6 reactivation [28] [33].

In patients with DCM, a possible pathophysiological role for circulating autoantibodies against cardiac proteins has been suggested [50] [53] [54]. Incremental research on this topic particularly in the past few years has significantly contributed evidence to the hypothesis that autoimmune reactions against certain myocyte antigens may play a crucial role in the initiation and/or progression of DCM. Transfer experiments in animals performed by various groups and some preliminary clinical data even indicate that a few of these autoantibodies are indeed pathogenic, inferring that they can actually cause cardiac dysfunction and heart failure by their own. Dependent on the individual genetic predisposition such harmful autoimmune reactions are supposed to emerge as a consequence of heart muscle damage induced by viral triggers, ischemia or exposure to cardiotoxins leading to myocyte apoptosis (and/or necrosis) and subsequent liberation of a critical amount of self-antigens previously hidden to the immune system [53].

8. Limited Value of Genetic Testing for Cardiomyopathies

The detection of a gene mutation responsible for a monogenic disorder is the greatest effort of genetic testing. Current guidelines recommend genetic screening (evidence level A) for ARVD/C, HCM and DCM with conduction abnormalities or extra cardiac manifestations [67]. The majority of these mutations were identified by family screening tests, where one or more members were suffering from identical cardiac problems or died at younger age for unknown reason. Most genetic and clinical testing for cardiomyopathies has the drawback of trusting on imaging techniques as primary cardiological diagnostics. A differential diagnosis of infiltrative processes of myocardium or wall hypertrophy in acute inflammatory diseases caused by edema is requested, because in biopsies of patients suspicious for genetically determined cardiomyopathies as HCM or ARVD a massive infiltration of inflammatory cells or viral genomes are often seen, undetectable by echocardiography, CMR or computer tomography (CT).

Recent studies have identified a massive overrepresentation of previously described cardiomyopathy-related genetic variants in a healthy control cohort with genotype prevalence thousand fold higher than in diseased patients [68]. This finding makes genetic testing without prior clinic-diagnostically definition questionable [26] [69].

In contrast, a 32-basepair deletion in the CC chemokine receptor 5 (CCR5, RANTES) gene (CCR5del32 polymorphism) was confirmed as a prognostic marker for acquired cardiomyopathies. This deletion leads to deficiency of this receptor for various proinflammatory cytokines. Homozygosity additionally results in reduced susceptibility to HIV infections [70]. This polymorphism is furthermore associated with an improved outcome in diabetes and coronary heart disease [71].

The long-term effect of the CCR5 genotype in patients with clinically suspected myocarditis or DCM revealed a reduced mortality of patients with a 32-basepair deletion of the CCR5 receptor. The data showed that CCR5del32 polymorphism is an independent genetic factor that influences outcome in patients with clinically suspected myocarditis and DCM, not associated with myocardial inflammation, diabetes or viral infections. As shown for CCR5, only endomyocardial biopsy supported genetic studies can identify genetic markers which are associated with clinical occurrence of genetically determined cardiomyopathies, while simultaneously excluding myocardial inflammation or microbial infections as a reason for preceding heart muscle disease [69] [72].

9. Biomarker Based Diagnostics for Personalized Medicine

Detection of monogenic causes for current presentation and long term prognosis of cardiomyopathies such as cardiotropic viruses, inherited base of viral infection (ciHHV-6) or CCR5del32 genotype are still limited but possible by measurement of only one diagnostic parameter. Diagnosis of complex mechanisms like individual immune response on different inflammatory or viral induced processes and thereon based treatment options request multiparametric determination of corresponding biomarkers.

Regardless of individual determined genetic background, nearly all multistep diagnostics could be accounted as part of personalized medical approach. After the first clinical confirmed hint by digital imaging techniques (echocardiography, CMR or CT) Morbus Fabry can be diagnosed by additional laboratory and cardiac enzyme tests or tissue biopsies. Morbus Fabry, confirmed by the reduced alpha-galactosidase activity of leukocytes, can be treated by enzyme replacement therapy. If amyloidosis has been proven histologically, further immunohistological classification of the corresponding subtype is required to distinguish an age-related from a genetically determined ATTR-form. The latter of which would require family counseling [59] [69].

Combination of currently available diagnostic findings in EMBs, with distinct immunohistological and virological profiles, support the selection of patients with active lymphocytic myocarditis or with circulating cardiac autoantibodies which will most likely benefit from immunosuppression as the optimized treatment decisions. Immunosuppression demands the exclusion of virus positive patients because these will deteriorate by application of immunosuppressive drugs [49].

Novel biomarkers such as miRNA or gene expression profiles of myocardial tissue will improve diagnosis of specific cardiac disease entities by overcoming biopsy-based sample error due to their non-focal expression pattern in the near future (**Figure 2**) [26] [37] [66]. They can be used for primary diagnosis but also for therapy monitoring, due to the fact that they change their profiles during therapy indicating an alteration of molecular markers at the focal or systemic level. All diagnostic biomarker should improve currently applied diagnostics for the exact diversification of cardiac patients which profit from proposed treatment options (**Figure 3**).

It is now possible to simultaneously assess the expression of tens of thousands of gene transcripts, providing a resolution and precision of phenotypic characterization not previously possible [73]. Multiparametric diagnostic systems, measuring differentially expressed genes or miRNAs, may thus become a valuable diagnostic tool of the future due to its high predictive value in respect to clinical course, treatment decisions and therapy monitoring [74].

Positive diagnostic findings in examined biopsies are confirming the myocardial condition, but negative results will not exclude infections, intramyocardial inflammation or other pathophysiological conditions. To reduce the biopsy-dependent sampling error an increased number of biopsies is required but could never overcome it completely. Therefore global and stable biomarkers to identify disease entities in the effected organ are urgently needed.

Of note, deregulation of myocardial gene expression is not limited to focal biopsy area. This allows the identification of specific disease situations by analyzing of neighbouring biopsies [75] [76]. Diagnosis of complex diseases by only one parameter is quite difficult hence current diagnostic biomarker panels are consisting of only few deregulated genes. With the help of novel biomarkers, viral infections or focal infiltration of inflammatory cells in myocardium can be diagnosed in neighbouring tissue samples without direct histological confirmation of these agents (**Figure 2**).

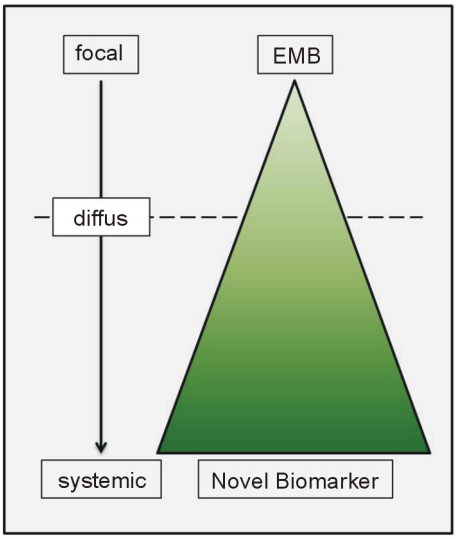

Figure 2. Increased diagnostic accuracy and reduction of biopsy-based (EMB) limitations by introduction of novel biomarker for personalized medicine of DCM patients.

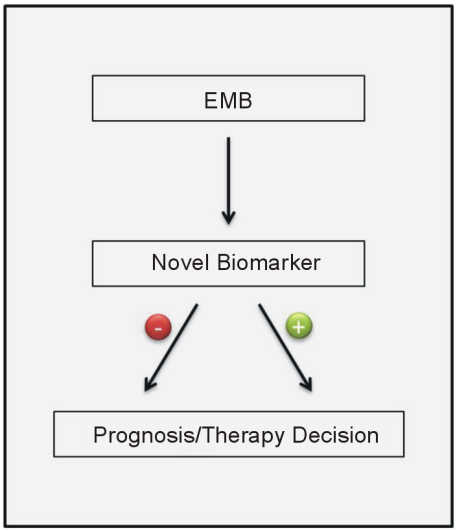

Figure 3. Biomarker support personalized treatment options after biopsy-based (EMB) diagnosis of different cardiomyopathies and various disease stages.

The identification and differentiation of IGCM and CS, two fatal myocardial diseases, is rather difficult and based on differential patterns of inflammatory cell infiltration and non-caseating granuloma. Since both multinuclear giant cells and granuloma are easily missed by conventional histological evaluation, an improved method to reliably identify those entities independent from histology is desirable [75] [76]. In this respect, the application of diagnostic gene expression profiling can predict multinucleated giant cells without their direct histological proof and thus differentiate IGCM from CS, active myocarditis or an inflammation-free myocardium [75]. According to the exact classification a personalized therapy can be administered (**Table 1**).

The application of immunoadsorption for removing circulating autoantibodies can significantly improve heart failure of patients with autoimmune DCM (**Table 1**). The selection of patients likely to respond to immunoadsorption has been established by comparison of the genetic expression profiles in samples from endomyocardial

Table 1. Personalized therapy options for different forms of myocarditis and DCM.

Disease	Subform	Applicable Drugs
Acute Myocarditis	Lymphocytic, Eosinophilic, Giant-Cell Myocarditis, Cardiac Sarcoidosis	Ciclosporin, Prednisolon
Chronic Inflammatory cardiomyopathy		Prednisolon, Azathioprin
Chronic Viral Cardiomyopathy	Enteroviruses, ADV, Parvovirus B19, HHV6A/B, CMV, EBV, HSV, VZV, Hepatitis C, RSV	Interferons, Val-/Ganciclovir, Ribavarin, Telbivudine, Cidovir, Aciclovir, Foscanet
Chronic Autoreactive Myocarditis		Immunoadsorption

biopsies of patients with DCM and controls [50] [53] [54]. Responders could be discriminated from non-responders by their differentially expressed genes in myocardial tissue [77]-[79].

As shown for enterovirus positive patients some patients are able to eliminate this virus spontaneously by intrinsic immune response with hemodynamic improvement and better long-term outcome [64]. Whereas patients with enterovirus persistence have an increased 10-year mortality [64] [65]. The use of novel biomarkers like gene expression or miRNA profiles allows the identification of enterovirus positive patients who will improve spontaneously by elimination of the virus, and those patients who will need a supportive interferon-β treatment (**Figure 3**) [64] [65].

The role of miRNAs in physiologic and pathologic processes and the capability to correlate expression changes with distinct disease states highlights their value as novel molecular biomarkers [80]-[82]. Currently available miRNA profiles allow the identification of preceding cardiotropic infection even in PCR-negative biopsies. The clinical course of such patients becomes predictable at the point of primary biopsy and a disease-specific therapy can be initiated immediately to prevent ongoing myocardial injuries [26] [64]-[66].

Symptomatic erythrovirus-associated heart disease may be caused by virus reactivation [26] [37] [66]. Measurement of characteristic signatures of mRNA and cardiac miRNAs allows the differentiation between latent and reactivated infection [37] [66]. Patients with transcriptionally active erythrovirus infection benefit from antiviral treatment (**Table 1**) [26].

Viral infection or intramyocardial inflammation is not only isolated to heart muscle tissue but also affecting the whole body as well. This process is attracting peripheral blood cells or inducing changes of cellular metabolism in other organs by soluble systemic mediators like cytokines or chemokines or releasing specific miRNAs in body fluids. Based on their high biostability, miRNAs are also present in body fluids such as serum, plasma or liquor protected from endogenous RNase activity by inclusion in exosomes or protein complexes. Molecular signatures of miRNAs are influencing the systemic response on organ-specific alterations (**Figure 4**).

Disease-specific miRNA signatures in tissues differ significantly from those in blood cells, serum or other body fluids of the same patient and therefore have their own specificity for the present condition [26] [74]. As shown for Takotsubo cardiomyopathy, a potentially life-threatening disease, which is clinically indistinguishable from acute myocardial infarction, circulating miRNAs are found which allow a differentiation of Takotsubo cardiomyopathy and myocardial infarction [83].

These findings emphasize the potential of circulating miRNAs as stable blood-based markers for the detection of cardiomyopathies. The investigation of miRNA expression profiles in body fluids opens a new diagnostic approach to detect and characterize focal diseases by systemic markers with an easily taken and abundant sample [26] [75] [76] [80]. Application of diagnostic miRNA profiling can improve EMB analysis by predicting the clinical course of disease, resulting in faster therapy decisions and monitoring of applied treatments. Therefore it could reduce the currently performed extensive workflow. Additionally, the signature of circulating miRNAs in blood or other body fluids will allow the diagnosis of organ-specific or systemic alterations by a minimal-invasive approach [74].

Beside diagnostics, the confirmation of differentially expressed genes or miRNAs, associated with various forms or states of cardiomyopathies are possible candidates for new therapeutic targets. In contrast to gene transcripts, the miRNAs are more favorable targets. Blocking of one miRNA influences one or more cellular pathways in the desired direction. Application of antagomir against translation of diagnostically applied biomarkers in pharmacologically usable drugs will broaden the currently existing treatment options [81].

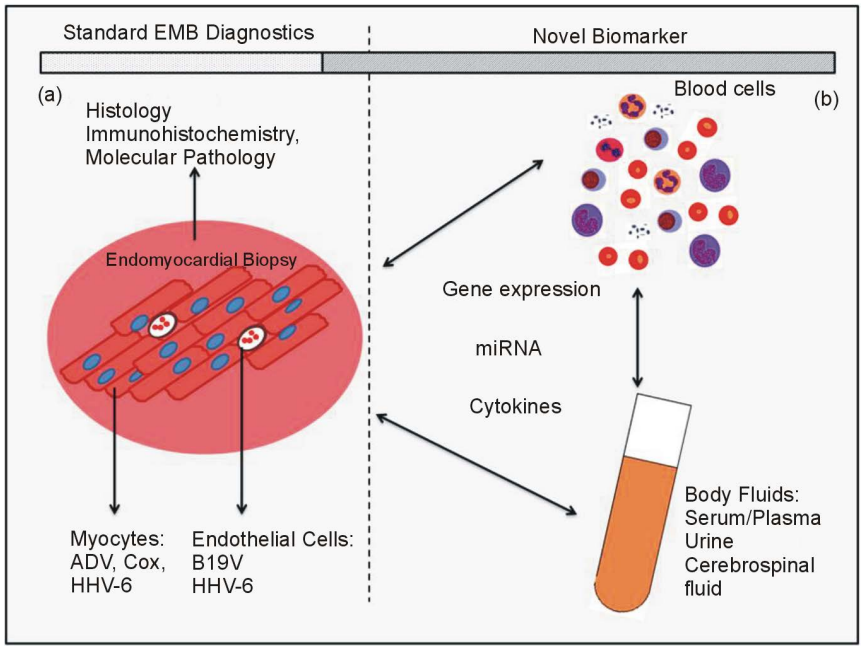

Figure 4. Novel biomarker like gene expression signatures and microRNA profiles improve current biopsy-based (EMB) diagnosis of different cardiomyopathies and various disease stages.

Nevertheless, the ultimate potential application of transcriptome-based molecular signature analysis is the individualization of the management of patients with heart failure, whereby an accurate assessment of prognosis and how individualized medical therapy could affect his or her outcome can be offered to patients with newly diagnosed cardiomyopathy [63] [73].

10. Future Perspective

General guidelines for diagnosis, treatment and corresponding quality analyses for myocarditis and acquired cardiomyopathies are still missing because unambiguous successful treatments proven by large randomized studies are rare. Positive outcome from treatment has only been established for some subgroups including enteroviral cardiomyopathy, giant cell myocarditis, sarcoidosis, or acute persisting acute-lymphocytic-myocarditis. One major problem is the identification of suitable patients for a distinct treatment option. In this regard, therapy guiding diagnostics must be standardized and supervised. This approach cannot be reached by clinical diagnosis but by biopsy-based information with complete histological, immunohistochemical, and molecular workup of tissue samples. Although EMB diagnostics are revealing only an extract of heart's current situation, this is still the gold standard for final confirmations of causative reasons of cardiomyopathies. The introduction of novel biomarkers like miRNAs or specific gene expression profiles as global organ-specific or systemic markers will reduce the EMB associated sampling error and will define an individualized therapy for each patient. This personalized approach allows an optimal application of available treatment options. Antiviral or immunomodulatory drugs (interferon, ganciclovir, telbivudine) improve survival or symptoms in the most frequent cardiotropic virus infections (adenovirus, enterovirus, erythrovirus and HHV6/ciHHV6) and immunosuppressive strategies are available for important infiltrative disorders such as chronic myocarditis, idiopathic giant cell myocarditis or cardiac sarcoidosis.

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