REVIEW

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Viral myocarditis: a prime example for endomyocardial biopsy-guided diagnosis and therapy

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Purpose of review
Myocarditis is an inflammatory disease of the cardiac muscle mainly caused by viral infection. Due to the diverse clinical presentation of myocarditis, accurate diagnosis demands simultaneous histologic, immunohistochemical and molecular biological workup of endomyocardial biopsies (EMBs) as defined by the position statement of the Working Group on Myocardial and Pericardial Diseases of the European Society of Cardiology on myocarditis.

Recent findings
Endomyocardial biopsy-based analysis of viral transcriptional activity, mRNA expression, epigenetics and region-specific protein expression analysis via imaging mass spectrometry have led to the identification of novel potential diagnostic criteria, markers with prognostic value and therapeutic targets for the treatment of viral myocarditis, opening new avenues for novel therapies, including cell therapies, as well as the use of established treatment options, be it from other indications.

Summary
Under certain clinical scenarios EMB-based analysis is required to come to a tailored individualized therapy that improves symptoms and prognosis of patients with acute and chronic viral-driven cardiac inflammation.

Keywords
coxsackievirus, endomyocardial biopsy, inflammation, parvovirus, viral myocarditis

INTRODUCTION

Myocarditis is an inflammatory disease of the cardiac muscle tissue caused by myocardial infiltration of immunocompetent cells following any kind of cardiac injury. Infectious causes include a vast number of viruses, bacteria, protozoa or fungi, but most frequently the myocardial inflammatory process is directed against viral pathogens. During the past decades, a shift is observed from adenoviruses and enteroviruses, including coxsackievirus B3 (CVB3) to parvovirus B19 (B19V) and human herpes virus 6 (HHV6) as the most frequently found cardiotropic viruses in endomyocardial biopsies (EMBs) [1–3]. Myocarditis leads to cardiac dysfunction and can progress to dilated cardiomyopathy (DCM). Patients with DCM have only a 5-year survival rate of 55% under current heart failure treatment, indicating the need for target-specific strategies [4]. A plethora of reasons speaks for the difficulty to diagnose and treat viral myocarditis: the diversity of the clinical presentation of myocarditis, the heterogeneity of the underlying cause, the difference in virus-specific target cell and pathogenesis, and the different disease stages following infection, indicating the need to combine clinical evaluation with in depth analysis of EMB, allowing characterization of the specific virus, and the amount and type of infiltrated immune cells [5]. The present review briefly outlines the relevance of the EMB for the diagnosis of viral

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KEY POINTS

- The EMB is the gold standard for the diagnosis of patients with suggested viral myocarditis who are in cardiogenic shock (class I recommendation) or do not recover over time despite conservative therapy (class IIa recommendation).
- An EMB-based exclusion of viral persistence permits the use of immunosuppressive therapies.
- IFN-ß might be a therapeutical option for EMB enterovirus/adenovirus-positive patients.
- Defined EMB-based analysis of viral transcriptional activity, mRNA expression, epigenetics and region-specific protein expression analysis via imaging mass spectrometry allows the identification of novel diagnostic and prognostic markers and therapeutic targets.
- EMB-guided diagnosis allows personalized therapies against viral myocarditis.

myocarditis. In this context, the use of imaging techniques as diagnostic tool for viral myocarditis is critically discussed. Furthermore, the review summarizes how 'nonstandard' EMB-based analysis [viral transcriptional activity, mRNA expression, epigenetics, and region-specific protein expression analysis via imaging mass spectrometry (MS)] has led and may further lead to the detection of novel potential diagnostic, prognostic markers and therapeutic targets for the treatment of viral myocarditis. Novel treatments options are discussed including established therapeutic interventions, which are currently used for other indications, and cell therapies including mesenchymal stromal cells (MSCs) [6,7] and the EMB-based cell product: CardAP cells [8–10].

Endomyocardial biopsy as diagnostic tool

Despite well known limitations giving rise to false-negative results (sampling error) if only low numbers (<8–10 samples) are taken, EMB is a safe diagnostic tool and up to date the gold standard for the diagnosis of (viral) myocarditis, as via histology, immunohistochemistry and viral diagnostics, it allows the quantification and identification of immune cell infiltrates, the proof of viral RNA and DNA presence, quantification of viral loads and confirmation of virus subtypes via sequencing [11–16] via imaging MS on EMB, region-dependent analysis of protein regulation is possible, which enables the differentiation between patients cohorts as already shown for the discrimination between patients with or without cardiac inflammation [5].

The landmark study from Kuhl *et al.* [1] revealed that genomes from cardiotropic viruses can be found in EMBs of 75% of patients with suspected myocarditis. B19V and HHV6 are detected in about 70% and 14–18% of EMB of patients with persisting symptoms of unexplained heart failure, respectively [1]. Furthermore, a chromosomally integrated form of HHV6 infection (ciHHV6) is detected in 0.8% of patients with myocarditis or DCM [17], whereas adenoviruses/enteroviruses are found in about 10% of the cases and associated with a reduced outcome if no spontaneous viral clearance occurs [18]. The prognosis of HHV6 and B19V persistence seems to be better or even not significantly impaired compared with this of adenoviral/enteroviral presence and mainly depends on the degree of the inflammatory response [19]. For the single-stranded DNA virus, B19V, the analysis of DNA copy number and VP1/VP2 RNA expression, representing transcriptional activity, is discussed to be important. The additional measurement of VP1/VP2 RNA expression seems to be required as B19V DNA can also be found in the heart of healthy patients [20,21] and in many other nonerythroid tissues including the liver, lung, skin and brain [22]. Therefore, the etiological role of B19V in the development of myocarditis and DCM still remains unclear, and it is considered that B19V might rather be a bystander than a cause of myocarditis. Only high copy numbers of B19V DNA are currently thought to be myocarditis-related [23], but these are rarely found in EMBs. Recent findings from a collective of 415 consecutive cardiac B19V-positive patients with clinically suspected cardiomyopathy indicate that a subgroup of myocarditis patients characterized by transcriptionally active cardiotropic B19V have an altered cardiac gene expression compared with control patients and myocarditis patients with latent B19V. Imaging MS from a single-patient use study illustrated a different protein muster between EMB of B19V mRNA-positive and B19V mRNA-negative patients (Fig. 1), further supporting the hypothesis that probably mRNA B19V is related to myocarditis [15].

In addition to the extent of the viral load [24,25] and transcriptional activity of B19V [15], other studies further suggest that the relevance of B19V in myocarditis and DCM depends on the presence of other cardiotropic viruses [24]. For all these measurements, EMB-based analysis is required.

The screening of microRNA (miRNA) in EMB may further help to diagnose viral myocarditis. Twenty-nine differentially regulated miRNAs were detected between patients with latent and reactivated B19V infection [26]. Furthermore, transcriptome mapping of CVB3 cardiomyopathy patients

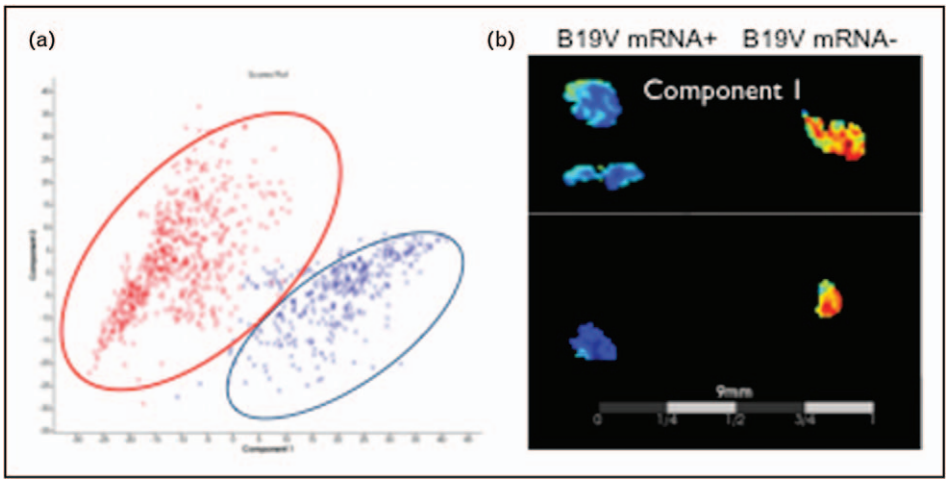

FIGURE 1. Imaging mass spectrometry enables the differentiation between parvovirus B19 mRNA-positive and mRNA-negative patients. In-situ tissue typing of endomyocardial biopsies in parvovirus B19-positive cardiomyopathy patients with low cardiac inflammation before (mRNA+) and after telbivudine treatment (mRNA−) via imaging mass spectrometry. (a) Principal component analysis distinguishes the protein signatures of the parvovirus B19 mRNA+ (red) and parvovirus B19 mRNA− patient group (blue). (b) Principal component analysis component I illustrates an increased intensity in distribution in parvovirus B19 mRNA+ versus parvovirus B19 mRNA− patients.

revealed distinctive cardiac miRNA patterns associated with spontaneous virus clearance and recovery versus virus persistence and progressive clinical deterioration, indicating the prognostic value of cardiac miRNA profiling to assess the risk of virus persistence and progressive clinical deterioration in CVB3 cardiomyopathy [27]. miRNAs reflecting cardiomyocyte injury, including miRNA-208 and miRNA-499, are increased in the blood of acute myocarditis [28]. However, these circulating markers are also upregulated in hypertensive cardiac disease and myocardial infarction (MI), indicating their unspecificity and inability to be used as diagnostic marker for (viral) myocarditis.

In general, the EMB cannot be replaced by viral serology, which often can lead to a false positive diagnosis. However, blood diagnostics allow to discriminate an acute viral infection from endogenous B19V or HHV6 reactivation, especially in cases with high virus loads, as occasionally detected in patients with HHV6 reactivation [29].

The measurement of laboratory parameters [high-sensitivity troponin T, N-terminal B-type natriuretic peptide, C-reactive protein (CRP)] alone are not sufficient for risk assessment in myocarditis. With the knowledge that persistent late gadolinium enhancement (LGE) is a risk marker of myocarditis [30], Berg *et al.* [31] recently conducted a study with the aim to evaluate whether routine laboratory parameters at diagnosis predict the dynamic of LGE by cardiac MRI. They found that cardiac enzymes and inflammatory parameters did not sufficiently reflect LGE in myocarditis and that by part of the patients with normalizing laboratory parameters, the LGE worsened. These findings stress that laboratory levels are too few for risk assessment in myocarditis and indicate that MRI might add value here too. Recently, LGE was found to be a risk marker even in patients with myocarditis and preserved ejection fraction [32,33].

Although imaging techniques including MRI can provide noninvasive tissue characterization and may localize large areas of inflammation, local and diffuse fibrosis, they cannot replace the EMB for the diagnosis of myocarditis due to the still high negative predictive value in acute as well as chronic settings [34], their inability to quantify inflammatory cell numbers and characterize the infiltrated immune cell subtypes and their incapacity to detect and quantify different virus types and loads [35]. In fact, a recent direct comparison of the EMB versus cardiac magnetic resonance in the MyoRacer-Trial [34] illustrated that in patients with acute symptoms, mapping techniques were a useful tool for confirming the diagnosis of myocarditis and superior to the Lake Louis Criteria, whereas T2-mapping had only acceptable diagnostic performance in patients with chronic symptoms, further supporting the EMB as gold standard for the diagnosis of viral myocarditis. Nevertheless, the indication to perform EMB remains controversial due to its invasiveness and false-negative results, especially in cases of focal pathological substrates [36]. Three-dimensional electroanatomical voltage mapping (EVM; e.g. CARTO system; Biosense Webster, Inc., Diamond Bar, CA) offers the potential to identify low-voltage

areas that correspond to regions with structural and functional changes [37]. Cardiac MRI including parametric-mapping can noninvasively characterize areas of inflammation, diffuse and focal fibrosis and wall motion abnormalities, identifying areas different from EVM [34]. EVM-guidance or MRI-guidance could therefore increase the sensitivity and specificity of the conventional EMB approach by reducing sampling errors and allowing a deeper insight of different (local) pathologies [38].

Endomyocardial biopsy for treatment decisions

The major benefit to perform EMBs in patients with suspected viral myocarditis and cardiogenic shock (class I; C recommendation) or no recovery of cardiac function over 3 months despite conservative therapy (class 2A; C recommendation) is to exclude a severe cardiac viral persistence [39]. This cannot be done by MRI. Exclusion of viral persistence allows the use of immunosuppressive therapy, known to improve cardiac function [40,41]. Immunosuppression is contraindicated in enterovirus and adenovirus-positive patients. These patients might profit from antiviral IFN-ß, which induces viral clearance [42,43]. With respect to HHV6, only HHV6 symptomatic patients with reactivated viruses may need antiviral treatment with, for example, ganciclovir. Such treatments may improve cardiac complaints and heart failure by suppressing transcriptional virus activities, but in most cases, they do not clear the virus from the myocardium [17]. In comparison with B19V infections, latent HHV6 infections are less frequently associated with an inflammatory process of the myocardium [44]. If inflammation is severe, experienced centres also use anti-inflammatory drugs in HHV6-positive patients, as the virus can anyway not be cleared, even by antiviral medications, and ongoing inflammation is the most important driver for a worse outcome [19]. A similar discussion started with respect to B19V DNA-positive patients, suggesting that B19V DNA presence represents a more innocent bystander finding. However, antiviral therapies in myocarditis patients are not established yet and should only be offered by experienced centres and/or on the basis of studies.

Endomyocardial biopsy for the search of therapeutical targets

Screening of EMBs from patients with acute myocarditis, dilated inflammatory cardiomyopathy and DCM showed that the expression of nucleotide-binding oligomerization domain-containing protein 2 (NOD2), a pattern recognition receptor which recognizes single-stranded RNA-like CVB3 [45], was upregulated in EMBs of cardiac CVB3-positive versus CVB3-negative acute myocarditis, dilated inflammatory cardiomyopathy, DCM and control patients. Furthermore, NOD2 expression was increased in CVB3-positive patients but not in patients with a persistence of other cardiotropic viruses like the double-stranded DNA virus HHV6 or the single-stranded DNA virus B19V [46], suggesting that coinfection with HHV6 or B19V found in some CVB3-positive patients was irrelevant for NOD2 signalling. The regulation of NOD2 expression was independent of the grade and modus of inflammation or cardiac function/remodeling. The importance of NOD2 in CVB3-induced myocarditis was in addition deducted from $NOD2^{-/-}$ mice, which were protected from the detrimental effects of CVB3 [47▪▪]. The potential clinical relevance of NOD2 further followed from the consistent pairwise drop of NOD2 mRNA expression between time point 1 and 2 in CVB3-positive patients who eliminated CVB3 and improved cardiac function over time, an effect which was only found with respect to IL-1ß in four out of six patients. Further studies have to show whether the analysis of NOD2 in EMB could be useful as an additional differential diagnostic marker for CVB3-induced myocarditis under clinical conditions.

Evaluation of EMBs from CVB3-positive versus CVB3-negative patients further showed that the danger associated molecular patterns (DAMPs)/alarmins S100A8 and S100A9 are higher expressed in CVB3-positive versus CVB3-negative patients. In addition, S100A8 and S100A9 expression dropped in CVB3-positive patients, who eliminated the CVB3 virus over time, which was associated with an improved clinical course [48▪▪]. The pathophysiological role of the DAMPs S100A8 and S100A9 in cardiac CVB3-induced myocarditis further followed from the observations that $S100A9^{-/-}$ mice exhibited an improved left ventricular (LV) function, associated with less cardiac infiltrates of neutrophils and monocytes, a reduced LV oxidative stress and CVB3 copy number compared with WT CVB3 mice. In contrast, intraperitoneal application of S100A8 in $S100A9^{-/-}$ CVB3 mice induced the CVB3 copy number and cardiac inflammation versus $S100A9^{-/-}$ CVB3 mice and resulted in an impaired cardiac function resembling the wild-type phenotype again [48▪▪]. The abovementioned data together with the existence of specific anti-S100A8/S100A9 compounds [49] indicate that these alarmins may represent a new potential avenue for the treatment of (CVB3)-induced myocarditis, and that S100A8 and S100A9 might be diagnostic biomarkers [50]. A previous study in experimental autoimmune myocarditis demonstrated that administration of S100A8/

S100A9 was protective [51], emphasising that the fate of S100A8/S100A9, depends on the disease and/or the stage of the disease, further stressing the need for personalized and EMB-guided evaluation.

Novel treatment options

Based on the abovementioned findings from EMB indicating the relevance of S100A8 and S100A9 [48▪▪] and NOD2 [47▪▪] in CVB3 myocarditis, the finding that S100A8 and S100A9 activate the Nod-like receptor (NLR) Family Pyrin Domain Containing 3 (NLRP3) inflammasome [52] and that NOD2 downstream signalling [45] involves the NLRP3 inflammasome and IL-1ß, the S100A9 inhibitor paquinimod and several drugs counteracting the NLRP3 inflammasome and/or IL-1ß signalling are potential promising therapeutics for the treatment of CVB3 myocarditis. The relevance of the NLRP3 inflammasome also beyond CVB3 myocarditis [53] indicates the potential of NLRP3-targeting therapies for myocarditis with other (viral) causes. Nevertheless, the EMB data [47▪▪] and the inverse correlation between IL-1ß and the antiviral IFN-ß [45] collaborate that particularly CVB3 myocarditis patients may profit from these pharmaca as antiviral strategy. Indeed, with respect to IFN-ß, it has been shown that solely patients with CVB3 profit from IFN-ß therapy [42] and not B19V patients, by which IFN-ß is associated with B19V DNA persistence [42,54]. However, it should be noted that IFN-ß was only evaluated in patients with B19V DNA and not with transcriptional active B19V. Therefore, the efficacy of IFN-ß in patients with transcriptional active B19V cannot be excluded.

Paquinimod

Paquinimod, an immunomodulatory compound preventing S100A9 binding to Toll-like receptor-4 and receptor for advanced glycation end products [55], has been shown to improve experimental osteoarthritis [56], systemic sclerosis [57] and atherogenesis in diabetes [58]. Its potential to treat viral myocarditis has not been evaluated so far.

Colchicine

Colchicine is an anti-inflammatory agent, traditionally used to treat gout [59]. Its main working mechanisms are the inhibition of neutrophil chemotaxis, adhesion and mobilization, the reduction in superoxide production and the inhibition of NLRP3 inflammasome activity and IL-1β production. In addition, colchicine has antifibrotic and endothelial-protective features [60]. Colchicine has recently been shown to be successful for the treatment of different inflammatory cardiac disorders, including stable coronary artery disease [61] and postpericarditomy syndrome [62]. The latter was successfully investigated in a placebo-controlled study. Most importantly, idiopathic (viral) pericarditis, triggered in 80% by typical cardiotropic viruses [63], inducing thereby often a peri/myocarditic response, was successfully treated with colchicine [64–66]. Colchicine is now a new European Society of Cardiology (ESC) recommended treatment option for different scenarios of pericarditis [67]. Therefore, colchicine is most likely effective in inflammatory cardiomyopathy too, as in the most cases, inflammatory processes as well as the role of cardiotropic viruses do not differ between pericarditis and myocarditis [63]. Indeed, a recent case report without controls indicated the efficacy of colchicine to treat patients with suggested myocarditis [68].

Anakinra

The relevance of the NLRP3 inflammasome in CVB3 myocarditis [69], its association with a worse prognosis on the long term [53] and the fact that IL-1ß is proteolytically activated upon NLRP3 activity [45,70] make the IL-1 receptor antagonist Anakinra an attractive candidate to treat viral myocarditis. Patients with fulminant myocarditis were successfully treated with Anakinra [71,72]. However, the potential of Anakinra to treat viral myocarditis has not been tested in clinical studies yet.

Canakinumab

Anti-inflammatory therapy targeting the IL-1β pathway with the fully human monoclonal antibody against IL-1β canakinumab led to a significantly lower rate of recurrent cardiovascular events than placebo, independent of lipid-level lowering in patients with previous MI and high high-sensitivity CRP levels ($\geq$2 mg/l) [73]. This successful effect of Canakinumab in preselected high-risk patients with high CRP levels supports the need to differentiate patient cohorts and to come to individualized therapies and collaborate its use for myocarditis patients with high CRP levels.

Mesenchymal stromal cells

MSCs are well known for their cardioprotective [74] and immunomodulatory properties [75]. In experimental settings of CVB3, we previously demonstrated that MSC also exert antiviral effects. Upon coculture with CVB3-infected HL-1 cells, MSC reduced the CVB3-induced apoptosis and consequent viral progeny release [6]. Furthermore, MSC decreased CVB3-induced CD4-T-cell and CD8-T-cell activation upon coculture with carboxyfluorescein

succinimidyl ester-labelled mononuclear cells. MSC exerted these antiviral and immunomodulatory effects in a nitric-oxide-dependent manner and required priming via IFN-γ. *In vivo*, intravenous MSC application improved the contractility and relaxation parameters in CVB3-induced myocarditis, which was paralleled with a reduction in cardiac apoptosis, cardiomyocyte damage, cardiac mononuclear cell activation [6], and cardiac fibrosis, and a moderate, but NS drop in CVB3 load [7]. In depth evaluation of the immunomodulatory properties of MSC under CVB3 conditions further revealed that heart and blood proinflammatory Ly6C^{high} and Ly6C^{middle} monocytes were reduced in CVB3 MSC versus CVB3 mice, whereas anti-inflammatory Ly6C^{low} monocytes increased in the blood, heart and spleen of MSC-treated CVB3 versus untreated CVB3 mice. In frame with the MSC-mediated modulation in monocyte migration towards the heart in CVB3 mice, LV expression of the chemokines CCL2/MCP-1 and MCP-2 attracting proinflammatory cells was reduced in CVB3 MSC mice, whereas LV stromal cell-derived factor-1α mRNA expression and systemic levels of fractalkine, known to attract anti-inflammatory cells [76], were increased in CVB3 MSC mice [77▪]. In a separate study, we additionally demonstrated that MSC limit cardiac and systemic NLRP3 inflammasome activation in CVB3 mice [78].

Regulatory T cells

Regulatory T cells (Tregs), a subpopulation of CD4$^+$ cells, constituting 5–10% of the peripheral T cells, and proclaimed as masters and regulators of the immune response [79], play a pivotal role in the induction and maintenance of immune homeostasis and tolerance in the setting of viral myocarditis [80]. Studies in myocarditis [81] and DCM [82] have shown that Tregs are quantitatively and/or qualitatively impaired under these conditions and consequently ineffective to balance the immune system. Therefore, direct Tregs application might be an attractive strategy to treat myocarditis, a hypothesis which is supported by experimental studies showing that prophylactic [81,83] and therapeutic [84] adoptive transfer of Tregs improves CVB3 myocarditis, and a strategy, which is feasible thanks to new technologies [85].

Telbivudine

Telbivudine is an antiviral nucleoside analogue reverse transcriptase inhibitor, which is especially effective for retroviral and pararetroviral (hepatitis B viruses) infections and has pleiotropic immunomodulatory/anti-inflammatory properties [86–89]. The single-stranded B19V DNA genome replicates through a specific rolling-hairpin-mechanism to generate a double-stranded DNA molecule mimicking DNA-synthesis during the reverse transcription process comparable with retroviruses and hepatitis B viruses [90]. As telbivudine preferentially inhibits the DNA-dependent single-stranded DNA synthesis, it is theoretically able to interfere with the unique replication mode of B19V, too. In a single-patient use, we could show an improvement of clinical symptoms, clearance of mRNA levels and changes in cardiac protein pattern after treatment with telbivudine in two B19V mRNA-positive patients with low cardiac inflammation (Fig. 1). This specific antiviral working mechanism of telbivudine together with its anti-inflammatory effects [86–89] have been the rationale to evaluate the efficacy of telbivudine in myocarditis associated with B19V transcriptional activity: the PreTopic Study (EudraCT-Number: 2016-004825-17).

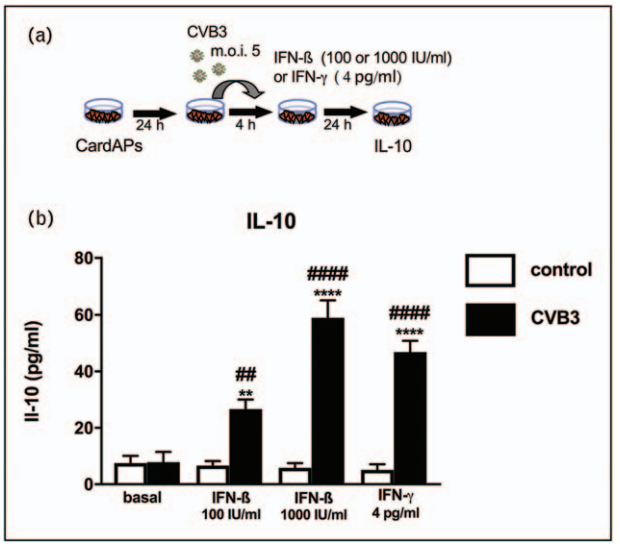

FIGURE 2. Impact of IFN-ß and IFN-γ on antiviral IL-10 expression of coxsackievirus B3-infected CardAPs. (a) Experimental design illustrating how CardAPs 24 h after plating were infected with coxsackievirus B3 at a multiplication of infection of 5 and 4 h after infection supplemented with/out 100 or 1000 IU/ml of IFN-ß or 4 pg/ml of IFN-γ. Twenty-four hours later, supernatant was collected for subsequent IL-10 analysis via ELISA. (b) Bar graphs represent the mean ± SEM of IL-10 in the supernatant of control CardAPs (open bars) or coxsackievirus B3-infected CardAPs (closed bars) supplemented with/out IFN-ß or IFN-γ, as indicated, with $n = 4–6$/group and $^{**}P < 0.01$ and $^{****}P < 0.0001$ versus respective control group and $^{\#\#}P < 0.01$ and $^{\#\#\#\#}P < 0.0001$ versus the basal coxsackievirus B3 group.

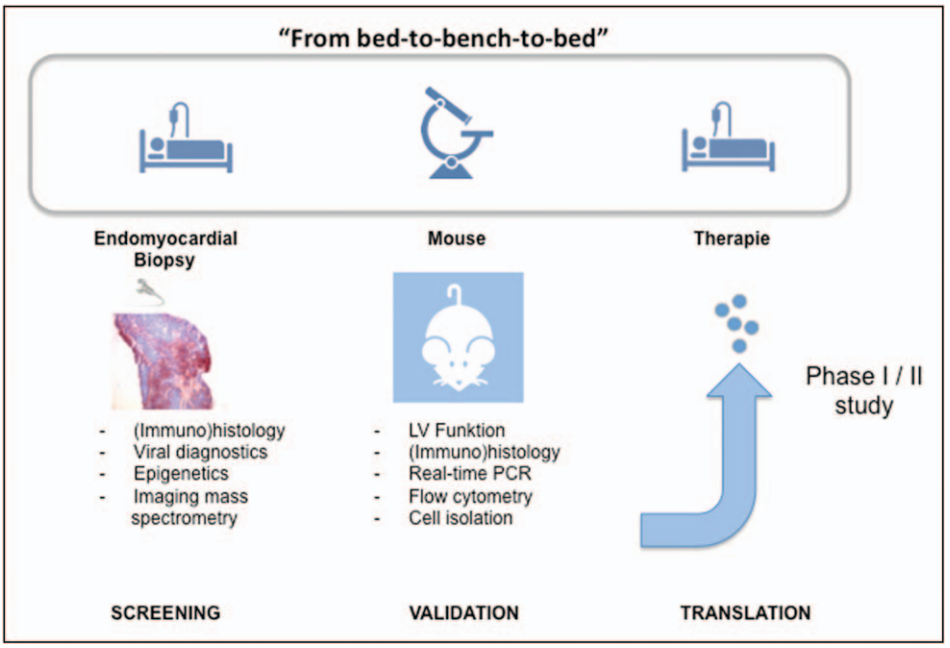

FIGURE 3. Bed-to-bench-to-bed strategy. Analysis of endomyocardial biopsies allows the identification of potential novel biomarkers and therapeutical targets, which are next validated in experimental mouse models. The efficacy of novel drugs will subsequently be tested in clinical Phase I/II trials (translation).

Endomyocardial biopsy as cell source

Significantly, EMBs can be used for the generation of so called Cardiac-derived Adherent Proliferating cells, CardAP cells [8]. Similar to MSC, CardAP have immunomodulatory [91], antiviral [9] and cardioprotective properties [10] and are able to reduce CVB3 viral progeny release in a nitric-oxide-dependent and IL-10-dependent manner. They also require IFN-γ to exert their antiviral effects. Intravenous application of CardAP cells in CVB3 mice led to an improvement in LV function, which was associated with a decrease in cardiac apoptosis, cardiac mononuclear cell activity, an increase in Tregs and T-cell apoptosis, and importantly, a reduction in cardiac CVB3 viral load [9]. In-vitro stimulation of CardAP cells with antiviral IFN-ß leads to the secretion of higher levels of antiviral IL-10 (Fig. 2), hereby further boosting their antiviral potential.

CONCLUSION

Viral myocarditis remains a major challenge in modern cardiology and underscores the need to explore innovative therapeutic options, which allow a sufficient antiviral defence with a balanced immune response preventing hyperactive inflammatory toxicity. The most recent position paper of the ESC working group on myocardial and pericardial disease stresses the need to search for novel biomarkers to improve diagnosis, prognosis and therapy of (viral) myocarditis [13]. EMB-based histological, immunohistological and molecular biological informations are prerequisites to establish an accurate diagnosis of viral myocarditis and successful management of patients and cannot be substituted by any noninvasive clinical analysis. Detailed EMB-based analysis has led to the identification of novel diagnostic, prognostic markers and therapeutics targets (Fig. 3), allowing differentiation of patients with viral myocarditis in smaller cohorts and mechanistically based individualized interventions.

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Conflicts of interest

There are no conflicts of interest.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

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