Review Article

Cardiovascular Involvement in Chronic Hepatitis C Virus Infections – Insight from Novel Antiviral Therapies

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Abstract

Whereas statistical association of hepatitis C virus (HCV) infection with cardiomyopathy is long known, establishment of a causal relationship has not been achieved so far. Patients with advanced heart failure (HF) are mostly unable to tolerate interferon (IFN)-based treatment, resulting in limited experience regarding the possible pathogenic role of HCV in this patient group. HCV infection often triggers disease in a broad spectrum of extrahepatic organs, with innate immune and autoimmune pathogenic processes involved. The fact that worldwide more than 70 million patients are chronically infected with HCV illustrates the possible clinical impact arising if cardiomyopathies were induced or aggravated by HCV, resulting in progressive HF or severe arrhythmias. A novel path has been opened to finally resolve the long-standing question of cause-effect relationship between HCV infection and cardiac dysfunction, by the recent development of IFN-free, highly efficient, and well tolerable anti-HCV regimens. The new direct-acting antiviral (DAA) agents are highly virus-specific and lack unspecific side-effects upon cardiac function which have always confounded the interpretation of IFN treatment data. The actual frequency of unexplained HF in chronic HCV infection will be determined from a planned large-scale study. Whereas such patients probably constitute a rather small fraction of all those harboring HCV, they have major clinical relevance. It is not yet known which fraction of these patients will significantly benefit from HCV eradication, but this issue will be addressed now in a prospective study.

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Introduction

Several studies have detected an association of hepatitis C virus (HCV) infection with cardiomyopathy, but no causal relationship or mechanistic link could be established so far.[1–7] Importantly, patients with advanced or pretransplant heart failure (HF) as defined by European Society of Cardiology (ESC) guidelines[8] are mostly unable to tolerate interferon (IFN)-based treatment regimes, resulting in very limited experience with this patient group regarding the possible pathogenic role of HCV infections. HCV infection often triggers disease in a broad spectrum of extrahepatic organs,[9–13] with innate immune and autoimmune pathogenic processes being involved,[14–16] and involvement of the myocardium in HCV-triggered autoimmunity would therefore not come as a surprise. There is no need to assume that HCV directly infects the myocardium, or that HCV impairs the function of a healthy heart and thus constitutes an independent cause of cardiomyopathy and HF. For HCV to have relevance for cardiovascular medicine it would already be sufficient that it indirectly disturbs cardiac function via an immune mechanism, and does so particularly in already injured hearts. In all cases, HCV elimination could result in functional improvement.

The fact that worldwide more than 70 million patients are chronically infected with HCV illustrates the possible clinical impact arising if cardiomyopathies were induced or aggravated by HCV.[14,17] If progressive HF or severe arrhythmias were induced in even a small fraction of all HCV-positive patients, this would still constitute a grave clinical problem. For this reason, it is most welcome that the long-standing hypothesis of cause-effect relationship between HCV infection and cardiac dysfunction may be conclusively tested now, enabled by the recent introduction of highly efficient and virus-specific direct-acting antiviral (DAA)-based anti-HCV regimens.[18–23] Cardiac functional effects of DAA-based HCV

Keywords: Autoimmunity; Cardiovascular immunity; Cardiomyopathies; Hepatitis C virus; Antiviral therapies.

Abbreviations: AdV, adenovirus; B19V, parvovirus B19; CAR, coxsackievirus-adenovirus-receptor; CI, confidence interval; CLDN1, claudin1; CMP, cardiomyopathy; CVB3, Coxsackievirus B3; CVD, cardiovascular disease; DAA, direct-acting antiviral; DAF, decay accelerating factor; EBV, Epstein-Barr virus; EGFR, EGF receptor; EMBs, endomyocardial biopsies; EphA2, ephedrine A2; ESC, European Society of Cardiology; HCV, hepatitis C virus; HF, heart failure; HHV6, human herpes virus 6; HTA, host targeting agents; HTX, heart transplantation; IFN, interferon; IRES, internal ribosome entry site; LDL-R, LDL receptor; NS, nonstructural protein; OCLN, occludin; OR, odds ratio; PBMC, peripheral blood mononuclear cell; RdRp, RNA-dependent RNA polymerase; SR-B1, scavenger receptor B1.

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elimination can be directly related to the infection, since the new DAA drugs are devoid of the often grave unspecific side-effects of IFN upon cardiac function. In general, the new regimens may broaden the spectrum of patients eligible for therapy. If these regimens confer significant benefit in cardiovascular or other conditions associated with HCV, extrahepatic HCV manifestations may become an indication for treatment even in the absence of significant liver disease.

Cardiovascular implications of chronic HCV infections

Whereas HCV is long known as the leading cause of hepatocellular carcinoma and cirrhosis,[10] the cardiovascular implications of HCV infections are incompletely explored and possible mechanistic links are essentially lacking. Multiple former studies regarding effects of HCV infection upon cardiovascular disease (CVD) risk produced ambiguous results. A recent meta-analysis of these studies[10] concluded that HCV-infected patients have increased CVD-related mortality (OR: 1.65, 95% CI: 1.07–2.56, p=0.02), carotid plaques (OR: 2.27, 95% CI: 1.76–2.94 p<0.001), and cerebrocardiovascular events (OR: 1.30, 95% CI: 1.10–1.55, p=0.002, total 7.611 cerebrocardiovascular events in the HCV group).

Association studies of HCV with cardiomyopathy[1–3,24] are, by nature, unable to resolve the clinical key question if there is true cause-effect relationship between HCV and cardiac dysfunction. Importantly, not even detection of HCV genomes or their replication[4] in endomyocardial biopsies (EMBs) can prove causality since HCV might still be an innocent bystander. Against the background of these methodological problems, detailed follow-up of cardiac function and morphology before and after DAA-based HCV eradication offers a very elegant and direct alternative to answer the question.

Using a DAA protocol, we recently found it well tolerated even in a patient with apparently end-stage HF.[24] A patient who was previously unable to tolerate IFN-based anti-HCV therapy as a consequence of grave cardiovascular and psychiatric side-effects had to be listed for heart transplantation (HTX) due inexorable cardiac disease progression. Since HCV-positive status generally excludes successful listing and significantly impairs transplant survival,[25,26] we subjected her to DAA treatment prior to anticipated HTX. Unexpectedly, she displayed rapid and stable improvement of her NYHA functional status from III-IV before to class II after HCV clearance, and HTX was no longer considered.[24] The conclusion that HCV was causally involved in her myocardial dysfunction is inevitable for two reasons. First, the DAAs used (ombitasvir, paritaprevir and dasubavir inhibiting viral NS5A protein, RNA-dependent RNA polymerase (RdRp) and NS3/4A protease, respectively, plus the CYP3A inhibitor ritonavir) will not eliminate known cardiotropic viruses (coxsackievirus B3 (CVB3), adenovirus (AdV), parvovirus B19 (B19V), human herpes virus 6 (HHV6), Epstein-Barr virus (EBV)) due to the different genome sequences and biological life cycles compared to HCV. Second, for none of the DAAs used a direct effect upon innate or cell-mediated immunity, or upon inflammation in general is known.

Whereas this is an encouraging observation, it is currently not known which fraction of all HCV-infected patients with advanced cardiac dysfunction will experience comparably strong therapeutic benefit from HCV clearance. A large-scale study is thus required to determine the true frequency of the combination of advanced HF with chronic HCV infection. Most likely, patients with severe cardiac dysfunction constitute a rather small fraction of all HCV-positive individuals only, but a subgroup of eminent clinical relevance. A study is therefore planned to recruit a sufficiently large number of HCV-infected patients with cardiac disease who are in need and eligible for state-of-the-art IFN-free HCV elimination therapy. Systematic cardiological follow-up will reveal to what extent and in which fraction of these patients HCV eradication does improve cardiac function. These patients need to be closely monitored for possible side effects (e.g., electrolyte disturbances)[24,27] since the DAAs are in general well tolerated by nonHF patients,[18–22] but experience with HF cohorts, especially with patients in advanced stages of HF, is lacking. There have been reports of serious bradycardia among patients treated with sofosbuvir and amiodarone, but systematic review and meta-analysis of pooled data from randomized controlled trials did not show an increased risk of cardiac outcomes.[28]

HCV virology and immunology and possible cardiac pathomechanisms

After the discovery of HCV and generation of infectious molecular cDNA clones in 1997,[29,30] HCV was classified as prototype *Hepacivirus* into the *Flaviviridae* family. HCV constitutes a diversified group of viruses classified into seven genotypes and multiple subtypes, circulating in those infected as continuously evolving quasispecies.[17,31,32] The same phenomenon, which is based on lack of proof-reading activity of the virus-encoded RdRp, is also observed for CVB3,[33] the prototype virus causing myocarditis and often resulting in dilated cardiomyopathy.[34–36] For both HCV and CVB3, continuous diversification of virus genome sequences has been documented.

HCV resembles CVB3 in another important molecular aspect. Both have positive-sense single-stranded RNA genomes which in the host cells serve directly as messenger RNA (Fig. 1B), and in association with modified cell membranes as template for replication through negative-strand full-length intermediates.[37,38] Both HCV and CVB3 employ internal ribosome entry site (IRES)-mediated translation and polyprotein processing of the long primary virus-encoded mRNA, and both use a particular type of polymerases designated as RdRp, which are important drug targets (Fig. 1B).

The molecular mechanisms of HCV and CVB3 replication therefore display important similarities, whereas tissue tropism and details of replication differ greatly. Cell surface receptors known to be involved in HCV and CVB3 attachment and internalization are depicted in Fig. 1A. With regard to tissue tropism, it should be noted that this need not be comprehensively determined by the normal receptor complement of target cells, i.e. CVB3 always being targeted to cardiomyocytes or HCV to hepatocytes only. Instead, breakdown of endothelial barriers or alterations of cell surface receptor expression induced by any disease[39–43] may lead to retargeting of a virus to organs and targets cells normally inaccessible to this specific virus. Thus, one report described the presence of HCV genomes in the myocardium[4] by direct analysis of EMBs.

Interferons as primarily host-targeting and rather unspecific therapeutic agents were used for virus suppression or elimination in HCV as well as CVB3 infections. The efficacy of IFN-γ was found to be high regarding virus elimination in CVB3 cardiomyopathy patients, resulting in less urgent clinical need for the development of CVB3-specific DAAs.[34,44,45] This is in sharp contrast to HCV elimination which could not be reached by use of IFN-based regimens, resulting in a high clinical need

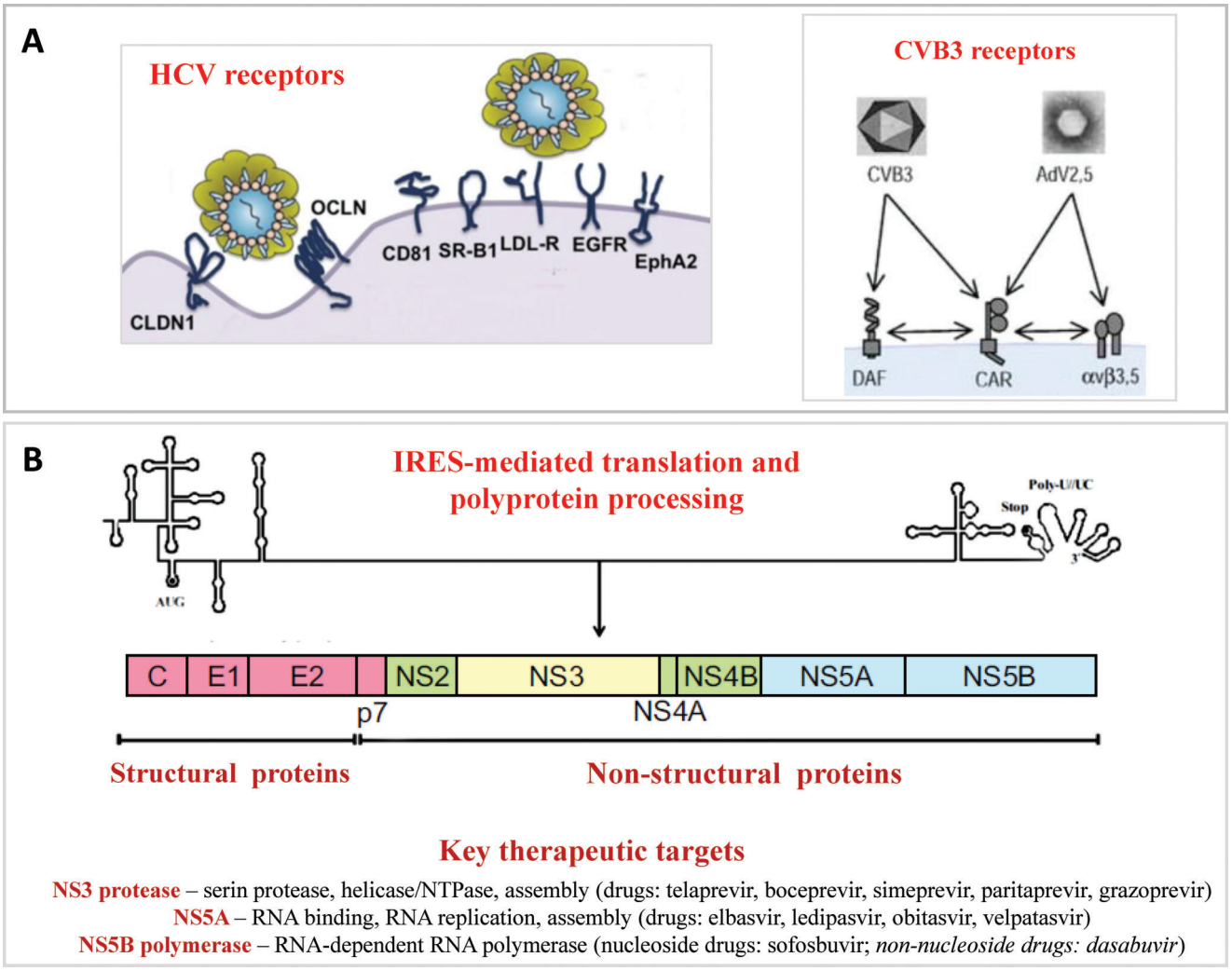

Fig. 1. Similarities between the life cycles of HCV and the prototypical cardiotropic virus CVB3. (**A**) Cell surface virus receptors determining the tissue tropism of HCV and CVB3. The molecular mechanisms of HCV and CVB3 replication display important similarities, whereas tissue tropism and details of replication differ. Those cell surface receptors known to be involved in HCV and CVB3 attachment and subsequent receptor-mediated endocytosis are depicted for HCV (left) and CVB3 (right), respectively. It should be noted that these receptors and coreceptors do not irreversibly determine the targeting path of HCV or CVB3 in the host, with CVB3 always reaching cardiomyocytes, and HCV hepatocytes only. Endothelial barrier breakdown or alterations of cell surface receptor expression induced by any disease[39–43] may lead to retargeting of a virus cell which is normally inaccessible to it.[4,39–43] (**B**) The complex intracellular viral genome transcription ultimately leading to HCV replication, and the key therapeutic targets addressed by DAAs. HCV constitutes a divergent group of viruses circulating as continuously evolving quasispecies.[17,31,32] The same phenomenon, which is based on lack of proof-reading activity of the virus-encoded RdRp, is also observed for CVB3,[33] the prototype virus causing myocarditis and often resulting in dilated cardiomyopathy.[34–36] For both HCV and CVB3, continuous diversification of virus genome sequences has been documented. The panel depicts that both HCV and CVB3 have positive-sense single-stranded RNA genomes which in the host cells serve directly as messenger RNA. Both employ IRES-mediated translation and polyprotein processing of the 9.6 kb primary virus-encoded mRNA, and both viruses use RdRp for this purpose. The primary 3000 amino acid polyprotein encompasses structural (C – nucleocapsid, E – envelope glycoproteins), as well as non-structural (NS) proteins required for polyprotein processing (NS3 protease) and viral genome transcription and replication (NS5B, RdRp). The RdRp of HCV emerged as an important drug target, which alongside drugs inhibiting the NS3 protease and the NS5A protein form the basis of current IFN-free HCV eradication protocols. Abbreviations: AdV, adenovirus; $\alpha_v\beta_{3,5}$, integrins; CAR, coxsackievirus-adenovirus-receptor; CLDN1, claudin1; CVB3, coxsackievirus-adenovirus receptor; DAF, decay accelerating factor; EGFR, EGF receptor; EphA2, ephedrine A2; HCV, hepatitis C virus; IRES, internal ribosome entry site; LDL-R, LDL receptor; NS, non-structural proteins; OCLN, occludin; RdRp, RNA-dependent RNA polymerase; SR-B1, scavenger receptor B1.

for DAAs against HCV. Another, clinically and economically important aspect of the far more intense research into anti-HCV DAAs as compared to anti-CVB3 drugs[46–51] is the fact that CVB3 cardiomyopathy is a rather rare disease,[52–60] whereas HCV infections are among the most frequent and important viral diseases worldwide.[23] Millions of patients are newly infected with HCV each year, chronicity rate is high, and over 70 million individuals are known to be infected.

According to current knowledge HCV replicates primarily, if not exclusively, in the patients' hepatocytes,[61] and its replication is strongly dependent on the liver-specific microRNA-122 which led to the development of a fundamentally novel anti-HCV therapeutic strategy based on an anti-miR-122 antagomir (miravirsen).[62] A number of other host molecules critical for HCV entry and replication were identified,[21] revealing important targets for the development of host targeting agents (HTAs). Although the use of miravirsen in patients with chronic HCV genotype 1 infection resulted in prolonged dose-dependent reductions in HCV RNA levels without evidence of viral resistance,[63,64] this path is no

longer followed and has been replaced by DAAs with their significantly higher efficacy and eradication potential. Likewise, none of the several anti-receptor strategies to block attachment and/or internalization of HCV[17] or CVB3 has so far proceeded to the stage of clinical evaluation.[31,34,65–70]

Whereas these studies have addressed host-related molecular mechanisms, other investigations of outstanding importance have addressed the structures and functions of essential HCV-encoded proteins, in particular three of those classified as nonstructural (NS).[17] The identification and characterization of

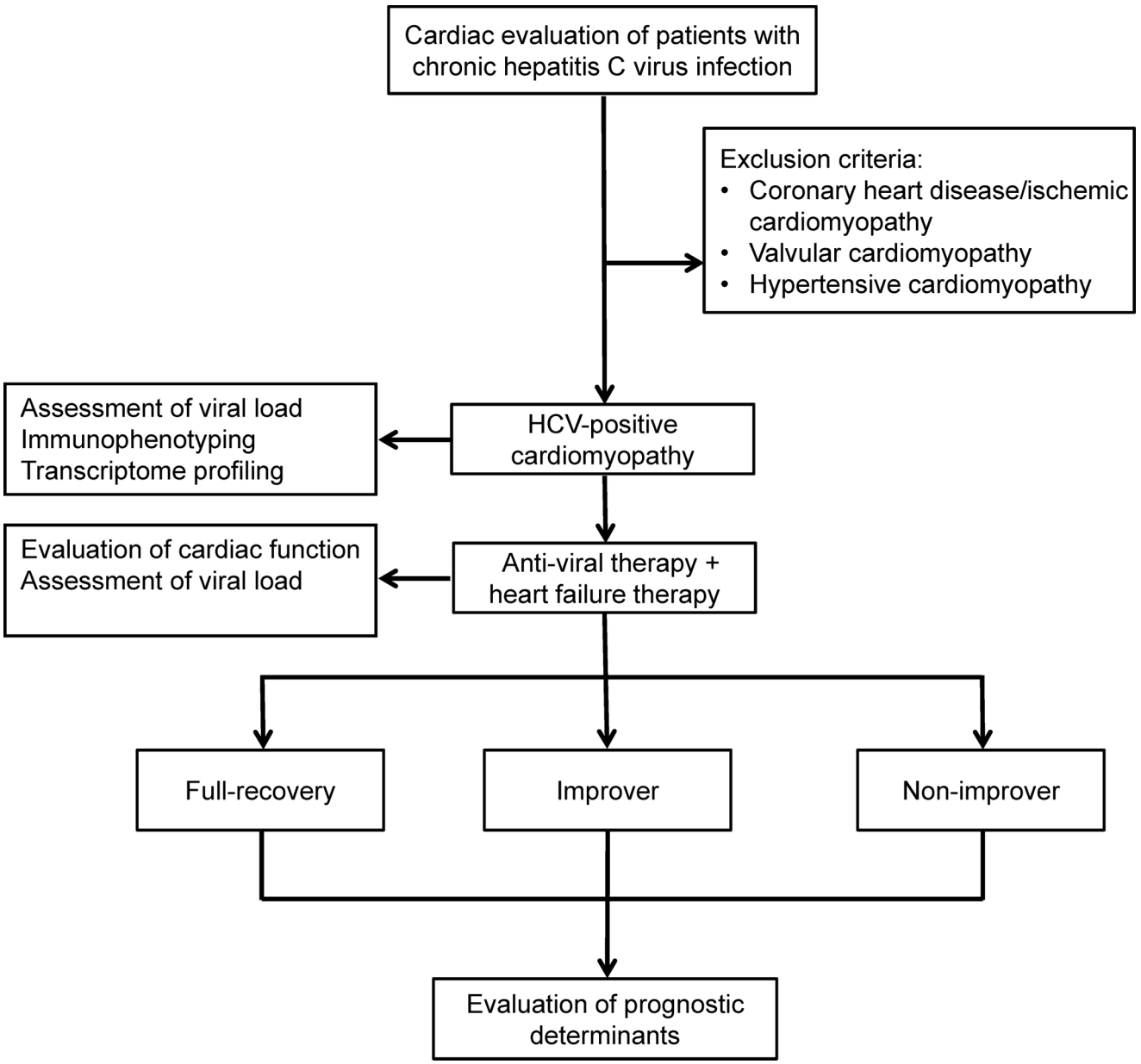

Fig. 2. Study to determine the impact of HCV eradication upon the course of myocardial diseases. The actual frequency of the combination of cardiomyopathy with chronic HCV infection is currently unknown. This study shall recruit a large number of HCV patients with myocardial disease who are in need and eligible for state-of-the-art HCV eradication.[15–18] Cardiological follow-up will reveal to what extent, and in which fraction of these patients, HCV eradication does improve cardiac function. For HCV to reach cardiovascular therapeutic relevance it would be sufficient that it indirectly disturbs cardiac function via immune mechanism, even if it does so only or particularly in already pre-injured hearts. Therefore, the study shall not only include patients with inexplicable left or right heart dysfunction or morphology, but also patients with nonvalvular and nonischemic cardiomyopathies, and pulmonary hypertension and/or right heart dysfunction of any cause. In these cases, HCV infection may adversely affect the "natural course" of the cardiac disease. The study is primarily based on noninvasive assessment (serial echocardiographies, HF biomarkers) to ascertain the frequency of combination of cardiomyopathy with HCV infection. In a subgroup of patients with advanced cardiac dysfunction[5] and/or or extensive morphological anomalies, right/left ventricular EMBs[68–70] are to be performed in accordance with ESC guidelines.[5] Patients are classified as IMPs if LVEF increases by 10 absolute percent units or if NYHA improves by one class. Patients are classified as NIMPs if they show at the follow-up visit any of the parameters such as an LVEF <35 %, failure to improve LVEF by 10 absolute units, remaining at a NYHA functional class of III/IV or obtaining heart transplantation/ventricular assist device or if patients die. Full recovery is defined as reaching an LVEF of >55 % and NYHA class I. Abbreviations: ESC, European Society of Cardiology; EMBs, endomyocardial biopsies; HCV, hepatitis C virus; HF, heart failure; IMPs, improvers; LVEF, left ventricular ejection fraction; NIMPs, nonimprovers.

HCV-encoded proteins and their functional units enabled the development of highly effective DAAs against the NS3 protease, NS5A and the NS5B polymerase of HCV. As already discussed above, IFN-free regimens based on these DAAs are not only far more efficient than IFN regarding HCV elimination, but they also have far less side effects. In combination, these DAA agents enabled IFN-free therapy with cure rates over 90% among patients with chronic HCV infection. Nevertheless, viral resistance represents a problem not yet fully solved.

Outlook from the cardiovascular perspective

Frequency of cardiac dysfunction in chronic HCV infection

One may safely assume that screening of retrospective series of HCV-positive patients for evidence of unexplained myocardial disease will only detect those with grave myocardial disease. Otherwise either the myocardial disturbance (e.g., isolated diastolic dysfunction) will go undetected, or in cardiological patients there are no data regarding possible HCV infection since there was no clinical hint for liver disease, and hence no apparent need for virus-specific diagnostics. The actual frequency of the combination of cardiomyopathy with chronic HCV infection therefore shall be determined in a prospective study (Fig. 2).

Cardiovascular therapeutic relevance of HCV elimination

In order to proceed beyond the question of frequency to possible cardiovascular therapeutic impact, this study has to recruit a sufficiently large number of HCV patients with cardiac dysfunction who are in need and eligible for state-of-the-art HCV eradication.[17–22] Careful cardiological follow-up will then reveal to what extent, and in which fraction of these patients, HCV eradication does in fact improve cardiac function. For HCV to reach cardiovascular therapeutic relevance it would suffice that it *indirectly* disturbs myocardial function via immune mechanism, even if it does so only or particularly in already pre-injured hearts. There is no need to assume that HCV *directly* infects the myocardium, or impairs the function of a healthy heart, thus constituting an *independent* cause of cardiomyopathy and HF. Therefore, the study shall not only include patients with inexplicable left or right heart dysfunction or morphology, but also patients with nonvalvular and nonischemic cardiomyopathies, and with pulmonary hypertension and/or right heart dysfunction of any cause. In these cases, the HCV infection might adversely affect the "natural course" of the cardiac disease.

Persistent immune system anomalies despite successful HCV elimination

The study is primarily based on noninvasive functional assessment of patients (serial echocardiography, HF biomarkers) which allows to ascertain the frequency of the combination of cardiomyopathy with HCV infection, and to address the question of cardiovascular therapeutic impact. In a subgroup of patients with advanced cardiac dysfunction[17–22] and/or or extensive morphological anomalies (e.g., massive left/right ventricular hypertrophy), right/left ventricular EMBs[71–73] are to be performed in accordance with ESC guidelines EMBs,[5] providing molecular virological data,[4,37,38] immunohistological data and immune-related gene expression profiles to identify and characterize inflammation, and histology to detect storage diseases. In addition, cardiac autoantibody arrays[74–76] will be conducted immediately before and 6 months after HCV elimination.

Several sets of data suggest that myocardial dysfunction and pathogenesis in chronically-infected HCV patients are immune-mediated. First, several studies have documented that autoantibodies to myocardial proteins including troponin I[74–76] and others[74–76] may aggravate cardiac dysfunction, and have prognostic relevance.[77–79] Virus infections induce an innate immune response not only when active viral replication takes place, but also in latent infections with even minimal viral synthesis of immunogenic nucleic acids or proteins.[5,80,81] In addition to this primordial innate immune activation, there may be virus-triggered autoantibody formation by molecular mimicry or other mechanisms. Second, it has been shown that DAA-induced HCV clearance does not completely restore the altered cytokine and chemokine milieu.[82–84] From the above immunhistological and serological characterization of the patients before and after virus elimination one may expect further insights into HCV-associated myocardial pathogenesis.

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

WP drafted the manuscript which was discussed and improved by all authors. The prospective HCV study described was derived from discussions among the authors all of whom will be involved in this project.

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