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Hepatitis C
From Infection to Cure

Edited by Imran Shahid

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Meet the editor

Dr. Imran Shahid is an assistant professor at Umm-Al-Qura University, Makkah, Saudi Arabia, where his research is focused on the broad areas of host–virus networks in hepatitis C virus (HCV) disease progression, as well as host–virus interaction during hepatic fibrosis, cirrhosis, and hepatocellular carcinoma. He is also enthusiastically involved in studies of HCV infection biology, cell signaling pathways, and genome heterogeneity in the Saudi population. He was trained at the Applied and Functional Genomics Laboratory, National Centre of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan, where he worked on anti-mRNA-based treatment strategies against hepatitis C in the Pakistani population. Dr. Imran has published many research articles in peer-reviewed international journals and is a member of the editorial boards of several international journals.

Contents

Preface

Hepatitis C virus (HCV) infection and associated hepatic comorbidities are still challenging, and disease burden remains significant around the world. Over the last decade, we have seen little curtailment in hepatitis C-related sequelae where the advent of direct-acting antivirals (DAAs) has clearly brought good news, as it may be possible to achieve a sustained virologic response—"a virologic cure"— in the majority of patients with chronic HCV infection. Clinicians are now seeing outcomes they never thought possible, and experts are optimistic that more complex and challenging patients will respond to therapy. All this has amalgamated efforts to purge the viral scourges, to cure the infection, and in parallel to accomplish the potential global goal of HCV eradication over the next few years.

Hepatitis C—From Infection to Cure is divided into four sections. The first section is the introduction, while second section sheds light on the propagation of HCV infection, molecular mechansims, and cell signaling cascades involved in the propagation of the disease. This section also presents HCV lymphotropism and its pathogenic relevance. The third section consists of chapters related to hepatitis C-associated clinical implications, especially periodontal implications and metabolic factors influencing the clinical course of hepatitis C and response to HCV treatment. The forth section overviews the current and emerging anti-hepatitis C regimens considering safety, tolerability, and associated side effects related to DAAs.

This informative book provides clinicians, physicians, healthcare providers, researchers, medical residents, and students with up-to-date, credible, and unbiased information/guidance for the treatment of hepatitis C, as well as knowledge of the latest developments and emergence of new treatment strategies to help stem infection. I am extremely grateful to all the contributors from different parts of the world who are excellent researchers, renowned clinicians/physicians, and emerging scholars in the field of hepatitis C virology, pathology, and therapeutics. I hope that their novel ideas and excellent scientific findings present a broad spectrum of academic and community-based knowledge of hepatitis C disease progression, associated clinical implications, and therapeutics to healthcare providers, nonspecialists, and general readers.

A journey is easier when you travel together and interdependence is certainly more valuable than independence. During this work I have been accompanied and supported by my colleagues at the College of Pharmacy, Umm- Al-Qura University, Makkah, Saudi Arabia and I am grateful for the opportunity to express my gratitude to all of them.

Finally, I dedicate this book to my father, Mr. Muhammad Hafeez , who is always with me at every step of my life, and all that I am or hope to be I owe to him, and my mother Tasneem

Sardar, as there is none in all this cold and hollow world, no fount of deep strong deathless love, save that within my mother's heart. I cannot finish without saying how grateful I am to my beloved wife, Miss Sidrah Hafeez, who provided me with a loving environment to accomplish this task and encouraged me to do my best in all matters of my scientific life.

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Introduction

Chapter 1

Introductory Chapter: Current and Emerging Anti-Hepatitis C Regimens: Hope or Hype

Imran Shahid

Additional information is available at the end of the chapter

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1. Introduction

1.1. Hepatitis C is curable now

Since its discovery in 1989, hepatitis C virus (HCV) infection always remained a big challenge for the physicians, clinicians, and healthcare providers to treat as well as for the scientists and researchers to design and develop novel compounds to inhibit viral replication and polyprotein processing. The classical treatment by interferon therapy as once known as the "gold standard of care" was not so much effective in different HCV genotype (GT)-treated patients, and treatment-emergent adverse events were generally a potential reason of treatment discontinuation [1]. The addition of ribavirin (RBV) to pegylated interferon (PEG-IFN) raised the hopes to achieve high cure rates; however, dual therapy was successful 70–80% in HCV GT 3 and only 50–60% in HCV GT 1- and GT 2-infected patients. In that era, the ample understanding of HCV life cycle, better understanding of pathophysiology of the disease, and the emergence of new technologies urged the researchers to develop novel compounds which directly target to different parts of the HCV genome which are essential components of viral replication and polyprotein processing [1].

1.2. Novel treatment options on the horizon

The landscape in hepatitis C medicine started to revolutionize from 2011, when the first time direct-acting antivirals (DAAs) were administered to hepatitis C virus-infected patients along with PEG-IFN and RBV [1]. After that, the advent and approval of different DAA combination have shifted the treatment paradigms for all seven HCV GT-infected patients and even difficult-to-treat-specific populations (HCV GT 1a- and GT 3-infected

individuals, cirrhotic patients, HCV/HIV coinfection, severe renal impairment and liver transplant, and previous treatment failure with NS3/4A or NS5A inhibitors). By inhibiting viral replication and blocking polyprotein processing, these novel and innovative DAAs are categorized into four groups, namely, nucleoside RNA-dependent RNA polymerase (RdRp; NS5B protein) inhibitors (NIs), non-nucleoside RdRp inhibitors (NNIs), viral replication complex inhibitors (i.e., NS5A protein), and viral serine protease (i.e., NS3/4A protein) inhibitors (PIs) [1]. Interestingly, the treatment regimens achieve higher sustained virologic response rates (SVR; HCV viral RNA undetectable at the end of week 12) only when used in combination as dual or triple therapeutic regimens, and single DAA therapy is either not so much effective or recommended to use [2]. Some DAAs have been developed as a single pill of fixed dose combination (FDC) of two, three, or even more compounds, and all DAAs are given orally to infected patients. The use of RBV is still considered an integral part of some DAAs while considering certain complicated hepatitis C patient populations including HCV cirrhotic patients; treatment-experienced patients with PEG-IFN, PEG-IFN/RBV, and first-generation PIs (telaprevir, boceprevir); and patients with decompensated cirrhosis. In parallel to that, the treatment strategies, dosage frequencies, and treatment duration vary for different HCV GTs and harder-to-treat-specific patient populations (e.g., HCV GT 3-infected patients; HCV patients with compensated/decompensated cirrhosis; previous treatment failure with PEG-IFN/RBV, first-generation PIs, and NS5A inhibitors; HCV/HIV coinfection; and patient with liver transplant or severe renal impairment). Some treatment options with pan-genotypic HCV coverage have been approved by the Food and Drug Administration (FDA) of the United States of America (USA), and some are in the final stage of development. Some different antivirals with an alternate mechanism of action such as by inhibiting viral entry or cell-to-cell spread and some anti-mRNA-based strategies like microRNAs are also in the pipeline [1, 2].

1.3. The treatment for hepatitis C is remarkably effective but with caveat

The clinicians, seeing outcomes they never thought possible, and experts are optimistic that more complex and challenging patients will respond to therapy. It has amalgamated the efforts to purge the viral scourges, to cure the infection, and to accomplish the potential global goal of HCV eradication. However, treatment choices can be tricky, and caveats are emerging including the recurrence of liver cancer and hepatitis B reactivation in chronically infected HCV or difficult-to-treat individuals. Equivocally, several important issues prevail linking to disease prevalence, viral screening, therapy adherence, reinfection, drug costs, expansion of care domain, and emergence of resistance-associated substitutions (RASs) in hepatitis C populations. Despite the incredible evolution in HCV therapeutics, much remains to be done to the point where it is a minimal entity. Now is the provident time to carefully consider population-level priorities and engineer HCV treatment strategies along with health policies which are realistic vis-à-vis implementation at a national or even pan-national level.

This book intends to comprehensively discuss hepatitis C virus infection progression, associated HCV clinical implications, and revolutionary anti-hepatitis C regimens and lends crucial insights on the opportunities that new therapies will bring to eliminate this silent but curable disease. It is not possible to discuss here HCV pathophysiology and therapeutics in detail,

but our intentions and aims in the introductory book chapter (Chapter 1) and the whole book itself are very clear: to provide general but valuable information from a common reader to an HCV specialist as well as to sketch a complete landscape of hepatitis C from infection to cure.

2. HCV disease progression

Afflicting around 170 million people worldwide [1], HCV infection is the foremost cause of liver cirrhosis, liver fibrosis, and hepatocellular carcinoma (HCC) and the first reason for liver transplantation [3]. The propagation of hepatitis C from acute to chronic infection and afterward to end-stage liver diseases involves a highly orchestrated series of molecular and cellular events including a plethora of genes and cell signaling cascades. The acute phase of infection is frequently asymptomatic or associated with mild and nonspecific symptoms. Persistent HCV infection is one of the major causes of chronic liver disease in 80% of infected individuals [4]. Approximately 20% of chronic carriers may develop liver cirrhosis, and some of these cases will progress to hepatocellular carcinoma (HCC). Consequently, HCV-induced chronic liver disease is recognized as the leading indication for orthotopic liver transplantation [5]. The studies demonstrate that HCV-induced inflammatory responses use host responses to recruit inflammatory cells and further prime a coordinated event of several host protein-protein interactions [4, 5]. Chapter 2 of this book highlights the recent advances in HCV-induced inflammatory responses and the role of inflammation during HCV infection. In addition, HCV affects other body cells including the immune system cells, and this ability of HCV to disrupt immune cells is evident to cause occult infection (e.g., mixed cryoglobulinemia and B-cell non-Hodgkin's lymphoma) [6]. How HCV lymphotropism affects the function of immune cells, virus persistence, and immune cell proliferation are discussed in Chapter 3. It is also believed that the progression of HCV to hepatocellular carcinoma (HCC) involved DNA methylation of cancer-related genes [7]. Chapter 4 illustrates how the detection of such genes may be helpful to know the different stages of disease progression from hepatitis C to HCC. In the setting of HCV infection, the role of various microRNAs (miRNAs) in modulating viral infection response has been deeply studied that clarifies causes of chronic hepatitis C progression in most infected patients and consequences of infection with manipulation in the risk of developing HCV-related comorbidities (i.e., cirrhosis and hepatocellular carcinoma) [8]. miRNAs are a class of small, endogenous, conserved, noncoding RNAs with a length of 20–24 nucleotides which posttranscriptionally regulate target genes. miRNA are also proved as key regulators of homeostasis for multiple biological systems, besides modulation of the disease pathology of many cancers. Similarly, miRNAs act as key modulators of HCV and hepatitis B virus (HBV) infection and liver disease progression [8]. Chapter 5 elaborates the regulation of miRNAs in HCC-related HCV patients.

3. HCV-associated clinical implications

Metabolic syndrome, obesity, and insulin resistance are very common health problems around the world with an increased morbidity rate. These comorbidities further contribute to hepatic steatosis, which ultimately lead to fat deposition in the liver [9]. Some studies predict that

these pathological states may limit response to IFN-based treatment regimens while treating hepatitis C; however, interestingly therapeutic response is not impaired to DAAs [10]. Now with curative treatment options available for patients with HCV, the sequelae of steatosis, fibrosis, and its drivers will garner more attention. Several other metabolic factors (e.g., vitamin D) could be related to more liver damage and high degree of fibrosis [9, 10]. In Chapter 6, Prof. Villar reviews the challenges and metabolic pathology associated with HCV infection and highlights some metabolic factors with their significant impact on liver damage. Several studies demonstrate that HCV infects other body organs and clinical implications may be very serious in chronically infected HCV patients. Some clinical studies suggest the association of HCV for the onset of periodontal disease in infected individuals [11]. The connection such connections between periodontal disease and hepatitis C must be considered by relevant healthcare practitioners due to their important implications on clinical manifestations and treatment strategies. Prof. Surlin Petra describes an update on periodontal implication of hepatitis C infection in Chapter 7. In addition to that, epigenetic modulation during HCV infection progression may also contribute to the development of HCV-related liver diseases (e.g., hepatic fibrosis and hepatocarcinogenesis) [12]. Chapter 8 describes the host and viral factors associated with the progression of hepatic fibrosis in HCV-infected individuals.

4. Current treatment landscape

4.1. The new HCV drugs are considered revolutionary

Treatment of chronic hepatitis C has markedly been improved with the introduction of IFN-free DAA therapies since 2011. New DAAs for chronic hepatitis C can cure infection in more than 95% of patients. Greater provision of DAAs, as well as greater efficacy of these medications in recent years, has led to a steady increase in SVR rates in HCV patients. The big picture is one of the clinical successes where we know that 95–100% of patients treated for hepatitis C can be cured [13]. It is pretty amazing.

The currently available DAAs are based on their target site with a particular mechanism of action [1, 2, 13]. NS3-4A serine protease inhibitors (NS3-4A PIs) block posttranslational processing of viral polyproteins by binding to the catalytic site of the enzyme which prevent the release of functional, nonstructural proteins. The first-generation PIs (i.e., telaprevir and boceprevir) were recommended for HCV GT 1-infected patients in 2011 in combination with PEG-IFN and RBV as a triple regimen with estimated SVR rates between 65 and 80%. However, the treatment-emergent adverse events, potential drug-drug interactions, essential necessity of PEG-IFN, and low genetic barrier to drug resistance were the major disadvantages associated with these drugs. Consequently these regimens are not recommended to treat hepatitis C and were discontinued. The landmark era in HCV therapeutics was started in December 2013 when the first IFN-free all-oral regimen, an NI inhibitor (sofosbuvir) also known as "Magic bullet," was approved for hepatitis C treatment [13]. The SVR rates achieved were more than 90%, and the regimen was considered safe, with fewer drug-drug interactions, very low adverse event profile, superior SVR rates, and an ability to use in combination with other regimens to treat difficult HCV populations with satisfactory therapeutic outcomes. After that a

series of innovative DAAs have been approved by the US FDA to treat hepatitis C with excellent SVR rates. The second-generation DAAs in combination are highly effective to treat the wide spectrum of HCV populations, and some have shown clinical promise as pan-genotypic and panfibrotypic coverages [2, 13]. The first-generation PIs include telaprevir (TVR) and boceprevir (BOC), while second-generation PIs include simeprevir (SMV), ritonavir-boosted paritaprevir (PTV), asunaprevir (ASV), grazoprevir (GZR), voxilaprevir (VOX), and glecaprevir (GLE). NS5A inhibitors block their regulation capability of viral replication within the replication complex and also inhibit the viral assembly and release. First-generation NS5A inhibitors include daclatasvir (DCV), ledipasvir (LDV), ombitasvir (OBV), and elbasvir (EBR), while velpatasvir (VEL) and pibrentasvir (PIB) are categorized into second- or next-generation NS5A inhibitors. NNIs bind to one of the four allosteric sites of RdRp and block the catalytic function of RdRp which indirectly block RNA replication. Dasabuvir (DSV) is the only FDA-approved NNI, which is used in combination with other DAAs to treat hepatitis C. NIs act as a false substrate for HCV RNA polymerase enzyme during viral replication where its incorporation results in chain termination during viral RNA synthesis. Sofosbuvir (SOF) is the sole example in this category of IFN-free DAA regimens. However, SOF sets a new standard of care for HCV patients as it is used in combination with other DAAs for the treatment of almost all HCV GT patients and even difficult-to-treat-specific populations [1, 2, 13].

4.2. Pan-genotypic regimens

Until now, the US FDA has approved three pan-genotypic DAA combination regimens to treat HCV GT 1–6 and even difficult-to-treat-specific populations. The first pan-genotypic combination regimen including SOF and VEL (*Epclusa*®) as a FDC of single pill for 12 weeks is recommended for GT 1–6 patients without cirrhosis or with compensated cirrhosis. The regimen is also administered to patients with decompensated cirrhosis; however, in this case RBV is added to active regimens [2]. In July 2017, the US FDA approved *Vosevi*® (SOF/VEL/VOX) to treat adults with chronic hepatitis C GT 1 to 6 without cirrhosis or with mild cirrhosis [14]. *Vosevi*® is a once-daily single tablet that contains two previously approved drugs—the NIs SOF (400 mg) and NS5A inhibitor VEL (100 mg)—and the newly approved pan-genotypic PIs VOX (100 mg). *Vosevi*® is the first FDA-approved treatment for patients who have been previously treated with the DAAs SOF or other drugs for HCV that inhibit NS5A. SVR12 was achieved in more than 90% of patients after the end of treatment [14]. In August 2017, the US FDA approved the combination of GLE and PIB (*Maviret*®) for the treatment of chronic hepatitis C for adults with chronic HCV GT 1 to 6 without cirrhosis or with mild cirrhosis including those with moderate to severe kidney disease and those on dialysis [15]. It is also indicated for adults infected with HCV GT 1 who were previously treated with either an NS5A inhibitor or an NS3/4A PIs, but not both. The drug reduces by 4 weeks the time needed for a cure by administering once daily as three oral tablets. The treatment regimen for GLE/PIB lasts 8 weeks, while the standard treatment length previously was at least 12 weeks for other DAA combinations. The combination GLE/PIB is also effective for treating HCV infection in individuals coinfected with HIV-1, according to results from the non-randomized, open-label phase III clinical trials [16]. Both *Vosevi*® and *Maviret*® are active against all HCV GTs, and with little differences, the two medicines may be specifically useful in some harder-to-treat-specific populations or those who failed or cannot use previously available therapies

[14–16]. In Chapter 9, Prof. Sidra discusses the safety, tolerability, and associated side effects of DAAs emphasizing their clinical pharmacology as well as the important safety issues of drug-drug interactions (DDIs). Similarly, Prof. Tran in Chapter 10 overviews a mathematical model while using sensitivity and identifiability techniques to determine model parameters in hepatitis C viral dynamics using a combination therapy of IFN, RBV, and TVR for partial viral response, sustained viral response, and breakthrough patients.

4.3. Emerging anti-HCV regimens

An 8-week regimen containing grazoprevir-ruzasvir-uprifosbuvir appears to be effective for treating hepatitis C virus infection in patients with or without cirrhosis, according to findings from a pair of randomized phase II open-label trials [17]. SVR12 rates with 8 weeks of therapy were 93% in individuals with GT 1a, 98% with GT 1b, 86% with GT 2 (without cirrhosis, patients with HCV GT 2 and cirrhosis received a longer course), 95% with GT 3 (treatment naive, without cirrhosis), and 100% with GT 4 and 6. Interestingly, the 8-week duration of therapy for HCV GT 2 patients achieved lower cure rates; however, treatment extension to 12 weeks overcame this effect. The excellent treatment outcomes in phase II clinical trials support further investigation of grazoprevir, ruzasvir, and uprifosbuvir as a pan-genotypic regimen in phase III clinical trials, where this combination has the potential to provide a safe, single-duration regimen for HCV patients with and without cirrhosis including harder-to-treat GT 3 individuals who had previously treated with PEG-IFN and RBV. The clinicians are also hopeful that excellent therapeutic outcomes of such regimens in ongoing phase III clinical trials will provide safer options with regard to pan-genotypic regimens for the treatment of hepatitis C and may impact the current treatment landscape [17].

5. Challenges for new regimens

The advancement in HCV therapeutics is fabulous and trustworthy after the introduction of well-tolerated and safe oral interferon-free DAAs in treatment strategies which provides compassionate treatment for HCV-infected patients to get cured and back to normal life. However, the next frontiers in front of researchers and clinicians are to coup certain challenges which may interrupt to achieve high cure rates in treated individuals and may be a potential cause of suboptimal SVR rates in difficult-to-treat subpopulations in real-world clinical practice. Until now, the real-world clinical data is not so much largely published/produced to make a clear understanding and interpretation of these obstacles; however, the treatment costs, risks of HBV reactivation and liver cancer recurrence, and the emergence of resistance-associated substitutions (RAS) are potential barriers which may prevent to achieve the global goal of HCV eradication [2]. Likewise, the dosage algorithms and safety profiles of such regimens in patients under age 18, in pregnant females, and end-stage liver disease and post-transplant patients are yet to be extensively elucidated [2]. The following section briefly overviews these harboring issues with some supporting clinically published data and also suggests some possible solutions in this prospect.

5.1. Drug costs and treatment access

The high therapy cost in the developing world or even in resource-replete nations and lack of treatment access in some areas where HCV is highly prevalent (e.g., in Egypt and some part of South Asia, where HCV is endemic) are major limitations of current anti-hepatitis C regimens [2]. An average treatment cost may be from 65,000 to 110,000 USD when brand-name therapies are administered to HCV-infected patients for a 12-week duration. Is it splurging at these prices to cure hepatitis C? The answer is certainly no. In the USA, most of the insurance companies have adopted a policy of "prioritizing coverage to those who need it the most," and some states are authorizing the treatment while using the extent of a patient's hepatic fibrosis (stage 3 or 4) or cirrhosis to cover the cost of HCV drugs. In some states, the decision is usually left to third-party payers. In Europe, the healthcare policy frequently bases the administration of hepatitis C therapy on fibrosis stage, so the patients with later-stage disease are often given preference. Although the use of HCV generics cut the cost as first data are encouraging in clinical studies, it is too early to comment on the clinical efficacy of these regimens, and the emergence of adverse events in treated individuals is not fully elucidated, and studies are going on. The brand-name drugs are comparatively cheaper in Europe than in the USA but still much expensive in India. The generic drug costs should be even lower to provide these regimens to individuals who need it the most. Such treatment with minimal diagnostic support is urgently required in low- and middle-income countries (12 out of 20 countries with the highest ratio of HCV prevalence are classified as low or lower-middle income) where treatment access is extremely limited due to high drug costs and complexity of patient management. In such areas, "test-and-treat strategies" and "risk-stratified approach" could be implicated to provide a targeted therapy for patients with high risks of HCV progression. In this context, risk prediction tools may help the physicians and patients to decide whether to initiate the treatment with costly DAAs or to sustain affordable treatment even with PEG-IFN and RBV to achieve high SVR rates [2].

5.2. HBV reactivation risk with DAAs

Patients with a past or current HBV infection can experience sometimes fatal HBV reactivation if they take any of 11 approved DAAs for hepatitis C virus (HCV) infection treatment [18]. The US FDA recommends a box warning for the drugs advising clinicians to screen patients for evidence of a past or current HBV infection before ordering antiviral treatment for HCV. The FDA identified 24 cases of HBV reactivation in coinfected patients treated with these antivirals from November 22, 2013, to July 18, 2016, in reports to the agency and published literature. Two patients died, and one needed a liver transplant. Interestingly, clinical trials for the HCV drugs in question/approval did not report HBV reactivation because they excluded patients infected with HBV and it was not reported as an adverse event in phase III clinical trials for the DAAs' approval. Similarly, such exclusion characterizes higher DAA safety, in terms of potential liver adverse reactions in the presence of one virus infection (i.e., HCV) instead of conducting more complicated safety evaluation of DAAs in patients infected with both HBV and HCV. The exact mechanism of HBV reactivation is still not known; however, it is considered that it may result from a complex interplay of host immunologic responses in the setting of infection with two hepatitis viruses. It is also assumed that HBV reactivation

may result from HCV clearance rather than a drug-specific toxicity. The treatment-induced reduction in HCV by DAAs also suppresses HBV, and the lack of activity against HBV of DAAs plus immunologic responses may escape HBV to reactivate [18].

Flare-ups of inactive or once-resolved HBV with DAAs have rung alarm bells before. In March 2016, the European Medicines Agency (EMA) announced that it had launched a review of six DAAs for HCV on the basis of reports of HBV reactivation in individuals infected with both viruses and who were treated with DAAs for HCV [19]. The Pharmacovigilance Risk Assessment Committee (PRAC) review covered six DAAs marketed in Europe for treatment of chronic HCV infection: daclatasvir (*Daklinza*®), dasabuvir (*Exviera*®), the combination of SOF and LDV (*Harvoni*®), simeprevir (*Olysio*®), sofosbuvir (*Sovaldi*®), and the combination OBV/PTV/r (*Viekirax*®). Since the start of this review, two other DAAs, the combination SOF and VEL (*Epclusa*®) and the combination EBR and GZR (*Zepatier*®), have been authorized in the European Union. In December 2016, the PRAC has confirmed the risk for hepatitis B virus (HBV) reactivation when DAAs are used for treatment of HCV infection. The PRAC recommends that, before starting treatment, all patients should be screened for HBV; patients found to be coinfected with HCV and HBV should be monitored and managed according to current clinical guidelines. Although the frequency of HBV reactivation appears low, the PRAC recommends that a warning be included in the prescribing information for these medicines [19].

In September 2016, the American Association for the Study of Liver Diseases and the Infectious Diseases Society of America (AASLD/IDSA) issued updated guidelines that advise clinicians not to prescribe DAAs to patients with HCV until the patients are screened for HBV, all because the societies were hearing about HBV reactivation in coinfected patients treated with the drugs. If patients who test positive for HBV warrant treatment, they should begin that treatment before or at the same time they start to receive direct-acting antivirals for HCV, according to the guidelines.

5.3. DAAs and cancer risk

5.3.1. Evidence pointing to a heightened cancer risk

DAAs do not appear to increase risk for liver cancer in patients with hepatitis C infection and cirrhosis, but the drugs could make existing but previously undetected cancers worse and harder to treat, according to results from a large-scale prospective study [20]. An interesting but unexpected finding of this study depicted that 50% of the individuals who developed a tumor early in the course of treatment or just after stopping treatment developed a more aggressive type of tumor than what was usually seen in the course of the disease. The researchers hypothesized that HCV replication is halted by DAAs; there are dramatic changes in the immunologic and molecular microenvironment in the liver and in tumor suppression mechanisms, which could allow or even promote the growth of previously undiagnosed microscopic HCC foci. Therefore, it is mandatory that patients treated with DAAs with advanced liver disease continue to be monitored for HCC. The findings also point to the need for careful pretreatment screening and continued monitoring of patients treated with direct-acting antivirals for hepatitis C in particular who have advanced fibrosis and are therefore at risk for liver cancer [20].

An Israeli study also points to an increased risk for malignancy in hepatitis C patients treated with DAAs [20]. Findings from the retrospective assessment of 273 consecutive patients infected with hepatitis C, some with a history of liver cancer and others without, were also reported. A sustained viral response at 12 weeks was achieved by 95% of the patients. However, over the next 15 months, 14 patients, or 5% of all participants, developed malignancy. Specifically, there were six cases of de novo HCC, three cases of recurrent HCC, four cases of extrahepatic cancer, and one case of intrahepatic cholangiocarcinoma. This study also correlates an association between DAA efficacy and malignancies progression with higher risk although the exact mechanism of this association is not known. However, the researchers assumed that the sudden impairment of the immune system may allow the growth of existing preclinical cancer clones [20].

5.3.2. Studies finding no elevated cancer risk

In contrast, investigators saw no increased risk for HCC in patients treated with DAAs in a retrospective study of 178 patients with hepatitis C infection and HCC who were candidates for liver transplantation [21]. The research showed that the cumulative incidence of recurrence over 1 year was lower in patients treated with DAAs before a diagnosis of HCC than in a control group of patients never treated with DAAs. However, when the antivirals were administered after a diagnosis of liver cancer, the risk for recurrence was similar in the antiviral and control groups, which suggests that prediagnosis antiviral therapy could be protective. However, this study was conducted in a different population—liver transplant patients on a wait list, where a statistically significant decrease in HCC recurrence ($P = .04$) was noticed when patients were administered with DAAs before a HCC diagnosis. When patients had a complete initial response to cancer therapy, DAA use did not significantly increase the transplantation wait-list dropout rate. The study results support the use of DAAs in patients on the transplant wait list with HCC who have achieved initial response to locoregional treatment [21].

A systemic review, meta-analyses, and meta-regression revealed no difference in the risk for HCC in patients treated with DAAs and those treated with IFN-based therapy [21]. This study conducted in Australia, involving 13,875 people from 26 studies on HCC occurrence and 15 studies on disease recurrence, explicited other culprits involved in recurrence of HCC following DAAs treatment. In fact, the investigators note other factors could explain the higher incidence of cancer. The study analysis showed that the shorter duration of follow-up and older age of participants rather than the treatment regimen could be responsible for higher incidence of HCC. On meta-regression, DAA therapy was not significantly associated with HCC occurrence (relative risk [RR], 0.7; $P = .6$) or recurrence (RR, 1.4; $P = .49$) [21].

In a Scottish study, the risk for liver cancer after sustained virologic response was not significantly different between patients treated with IFN-free therapy and those treated with IFN-based therapy [21]. Of the 857 cirrhotic patients treated at one of 12 clinics in Scotland, 32% were treated with DAA regimens. During a median follow-up of 1.8 years, fewer patients in the interferon-free group than in the interferon group developed HCC (12 vs. 34). Even so, the risk was significantly higher in the IFN-free group (incidence rate ratio [IRR], 2.18; $P = .03$) [21].

5.3.3. DAAs cut risk of liver cancer

Eradicating hepatitis C with DAA therapy reduces the risk of HCC by 71%, according to results of a large observational study [22]. The findings are based on 62,051 patients who underwent 83,695 antiviral treatment regimens in the VA Puget Sound Health Care System. The data included 35,873 IFN-only regimens, 26,178 DAA regimens with or without IFN, and 21,644 DAA-only regimens. The researchers identified 3271 new cases of liver cancer diagnosed at least 180 days after the start of antiviral treatment during an average follow-up of 6.1 years. The incidence of liver cancer was highest in patients with cirrhosis who failed treatment (3.25 per 100 patient-years)—followed by patients with cirrhosis and sustained virologic response, or SVR, (1.97), no cirrhosis and treatment failure (0.87), and no cirrhosis and SVR (0.24). In multivariable models adjusted for potentially confounding factors, SVR was associated with a significantly reduced risk of liver cancer, regardless of whether the antiviral treatment was DAA-only (adjusted hazard ratio (aHR), 0.29), DAA with IFN (aHR, 0.48), or IFN-only (aHR, 0.32). In both cirrhotic and non-cirrhotic patients, the risk of liver cancer was reduced [22].

5.4. Viral resistance

The huge genetic diversity due to poor fidelity of its replication enzyme (i.e., RdRp) and rapid replication rate configures HCV genome into 7 distinct GTs, more than 84 subtypes, and even exists as a quasispecies in a single-infected patient [2]. The viral genome differs by >30% at GT level, >15% at subtypes level, and <15% within a specific GT (i.e., quasispecies) in an infected individual. This genome variation by nucleotide substitutions/mutations is considered a major reason for the origination of pre-existing or treatment-emergent RASs in DAA-treated patients [2, 23]. Baseline polymorphism and pre-existing or treatment-emergent RASs are the most considerable points to the physicians while deciding to initiate oral IFN-free DAAs for hepatitis C treatment in treatment-naive (TN) or treatment-experienced (TE) or treatment-failure patients [23]. It is a well-established fact that viral mutations make the virus less susceptible to treatment; and in the case of HCV, it has been proven that monotherapy will result in selection of mutations which enhance replication in the presence of a drug. HCV resistance occurs when nucleotide substitutions randomly appear throughout the genome with every replication cycle. Some nucleotide substitutions by chance intervene to bind specific DAAs to their specific protein target [23].

Viral variants with RASs in the presence of DAAs possess a fitness advantage, but in the absence of DAAs, most will be outcompeted by a wild-type virus [23, 24]. The viral fitness of specific RASs potentially determines whether RASs persist after unsuccessful DAA therapy and whether they exist at baseline in TN patients. Interestingly, RASs to different DAA classes express markedly different viral fitness. The knowledge of RAS may influence clinical management of HCV in terms of alter duration of therapy, to add RBV in specific difficult-to-treat subpatient populations and severity of disease (cirrhotic vs. non-cirrhotic) and to choose particular DAA regimen for retreatment. Interestingly, some RASs affect the treatment response to all members of a specific DAA class, whereas others have variable impact on different DAAs of the same class. Meanwhile, the prevalence and effects of RASs vary in different populations (i.e., within different HCV GT/subtypes and quasispecies, individual RASs may differ in emergence and differently impact SVR rates) and may be more relevant in TE patients and those with cirrhosis [23, 24].

Unfortunately, still there are no clear guidelines for resistance testing. Should it be a universal testing of all patients' pretreatment or selective? As we have seen that SVR rates approach almost 100% for most HCV-treated patients while administered to most DDA regimens [24], it is difficult to do for everyone. However, this approach is only applicable to test those patients where knowledge of the findings may influence clinical management although which patients are those is a big question [23]. AASLD in 2017 recommends NS5A RASs testing for LDV/SOF and EBR/GZR combination prior to initiate therapy among GT 1 patients by virus subtype, prior TE, and cirrhosis status [23]. For GT 3, RASs detection is recommended for SOF/DCV or SOF/VEL combination and both for TE and cirrhotic patients, and if Y93H is present, weight-based RBV is added to active regimens. In contrast, current EASL recommendations demonstrate that access and affordability to reliable HCV resistance testing are limited, and there is limited consensus on the techniques used, data interpretation, and reporting of these detections. Surprisingly, EASL does not enforce HCV resistance testing prior to treatment and applies only to TE patients who were previously treated with PEG-IFN/RBV, PEG-IFN/RBV/SOF, SOF/RBV, etc. [24].

6. Conclusions

The present is pretty great, and the future is extremely positive after the advent and approval of IFN-free DAAs to cure hepatitis C. We will continue to push boundaries and now are at a point that we should be able to eradicate hepatitis C with these drugs. The journey started from an NS5B inhibitor (sofosbuvir) to develop pan-genotypic regimens that offer new perspectives in HCV screening and medicine. One size does not fit all; however, in the case of HCV, we are at the edge of brick where a single pill could be effective for all HCV genotype-infected populations in the near future. Thus new therapies afford public health policy makers great opportunities but, equally, pose dilemmas too where their cost has sparked much controversy and debate over who should get them, and HCC recurrence and HBV reactivation are key obstacles preventing to achieve global goal of HCV elimination. With curative treatment options available for patients with HCC and HBV, linkage to care and adherence to screening/surveillance guidelines should be clearly warranted for early diagnosis of HCC and HBV. Now, our aim should be to stimulate discussion as to how we can capitalize on the opportunities that new therapies will bring in terms of their expected population-level impact and engineer our treatment strategies accordingly. Overall, the future of HCV therapeutics seems bright and becomes brighter every day as treatment combinations continue to be designed, developed, and approved.

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References

[1] Shahid I, ALMalki WH, Hafeez MH, Hassan S. Hepatitis C virus infection treatment: An era of game changer direct acting antivirals and novel treatment strategies. Critical Reviews in Microbiology. 2016;**42**(4):535-547. DOI: 10.3109/1040841X.2014.970123. Epub 2014 Nov 6

[2] Shahid I, AlMalki WH, Hassan S, Hafeez MH. Real-world challenges for hepatitis C virus medications: A critical overview. Critical Reviews in Microbiology. 2018;**44**(2):143-160. DOI: 10.1080/1040841X.2017.1329277. Epub 2017 May 25

[3] Feld JJ, Hoofnagle JH. Mechanism of action of interferon and ribavirin in treatment of hepatitis C. Nature. 2005;**436**:967-972. DOI: 10.1038/nature04082

[4] Alter HJ, Seeff LB. Recovery, persistence and sequelae in hepatitis C virus infection: A perspective on the long-term outcome. Seminars in Liver Disease. 2000;**20**(1):17-35

[5] Fishman JA, Rubin RH, Koziel MJ, BJ P. Hepatitis C virus and organ transplantation. Transplantation. 1996;**62**(2):147-154

[6] Pham TNQ, Coffin CS, Michalak TI. Occult hepatitis C virus infection: What does it mean? Liver International. 2010;**30**:502-511. DOI: 10.1111/j.1478-3231.2009.02193.x

[7] Zekri AR, Hafez MM, Bahnassy AA, Hassan ZK, Mansour T, Kamal MM, et al. Genetic profile of Egyptian hepatocellular-carcinoma associated with hepatitis C virus Genotype 4 by 15 K cDNA microarray: Preliminary study. BMC Research Notes. 2008;**29**(1):106. DOI: 10.1186/1756-0500-1-106

[8] Ura S et al. Differential microRNA expression between hepatitis B and hepatitis C leading diseases progression to hepatocellular carcinoma. Hepatology. 2009;**49**(4):1098-1112. DOI: 10.1002/hep.22749

[9] Cannon CP. Mixed dyslipidemia, metabolic syndrome, diabetes mellitus, and cardiovascular disease: Clinical implications. The American Journal of Cardiology. 2008;**102**:5-9. DOI: 10.1016/j.amjcard.2008.09.067

[10] Wong RJ, Gish RG. Metabolic manifestations and complications associated with chronic hepatitis C virus infection. Journal of Gastroenterology and Hepatology. 2016;**12**:293-299

[11] Maticic M, Poljak M, Seme K, Brinovec V, Meglic-Volkar J, Zakotnik B, et al. Detection of hepatitis C virus RNA from gingival crevicular fluid and its relation to virus presence in saliva. Journal of Periodontology. 2001;**72**(1):11-16. DOI: 10.1902/jop.2001.72.1.11

[12] Jovanovic-Cupic S, Kokanov N, Petrovic N, Krajnovic M, Kozik B, Kojic M, et al. The influence of baseline viral and host factors on therapy response in patients with chronic hepatitis C genotype 1b from Serbia. In: First Congress of Molecular Biologist Congress of Molecular Biologists of Serbia with International Participation; 20-22 September 2017; Belgrade, Serbia. p. 135

[13] Imran Shahid, Waleed H. AlMalki, Mohammed W. AlRabia, Muhammad H. Hafeez and Muhammad Ahmed. Hepatitis C virus infection treatment: Recent advances and new paradigms in the treatment strategies, Advances in Treatment of Hepatitis C and B, Prof. Naglaa Allam editor. Rijeka, Croatia: InTech; 2017. DOI: 10.5772/65873. Available from: https://www.intechopen.com/books/advances-in-treatment-of-hepatitis-c-and b/hepatitis-cvirus-infection-treatment-recent-advances-and-new-paradigms-in-the treatment-strategies

[14] FDA Clears Pan-Genotypic *Vosevi* for Chronic Hepatitis C. Available from: https://www.medscape.com/viewarticle/883095 [Accessed: 15-08-2018]

[15] FDA OKs 8-Week *Mavyret* for Hepatitis C. Available from: https://www.medscape.com/viewarticle/883778 [Accessed: 25-07-2018]

[16] Glecaprevir/Pibrentasvir Treats Hepatitis C in HIV-Coinfected Individuals. Available from: https://www.medscape.com/viewarticle/894696 [Accessed: 23-08-2018]

[17] Shorter Anti-HCV Regimen Effective in Patients With or Without Cirrhosis. Available from: https://www.medscape.com/viewarticle/884478 [Accessed: 09-08-2018]

[18] HCV Drugs May Reactivate HBV, FDA Warns. Available from: https://www.medscape.com/viewarticle/869784 [Accessed: 15-07-2018]

[19] EMA to Review New Hep C Drugs for Possible Hep B Reactivation. Available from: https://www.medscape.com/viewarticle/860609 [Accessed: 17-07-2018]

[20] Direct-Acting Antivirals for Hep C Might Worsen Liver Cancer. Available from: https://www.medscape.com/viewarticle/871831 [Accessed: 20-07-2018]

[21] Navigating the Hep C Treatment and Cancer Risk Minefield. Available from: https://www.medscape.com/viewarticle/881041 [Accessed: 06-08-2018]

[22] Direct-Acting Antivirals Cut Risk of Liver Cancer. Available from: https://www.medscape.com/viewarticle/887434 [Accessed: 28-07-2018]

[23] Pawlotsky JM. Hepatitis C virus resistance to direct-acting antiviral drugs in interferon-free regimens. Gastroenterology. 2016;**151**:70-86. DOI: 10.1053/j.gastro.2016.04.003

[24] Shahid I, Ibrahim MM, Nawaz MU, Imam MT, AlMalki WH. Resistance-associated substitutions/variants correlate to therapeutic outcomes of novel direct-acting antivirals in different HCV genotype treated individuals. In: Abdurakhmonov I, editor. Genotyping. Rijeka, Croatia: IntechOpen; 2018. DOI: 10.5772/intechopen.76395. Available from: https://www.intechopen.com/books/genotyping/resistance-associated-substitutions-variants-correlate-to-therapeutic-outcomes-of-novel-direct-actin

Section 2

Hepatitis C Virus Infection Progression

Chapter 2

Hepatitis C Virus and Inflammation

Binod Kumar, Akshaya Ramachandran and Gulam Waris

Additional information is available at the end of the chapter

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Abstract

Inflammation is often a rapid coordinated response generated in the host against evading microbial infections or tissue injury. Microorganisms like bacteria and viruses instigate inflammation mediated by pro-inflammatory cytokines and activate cascade of signaling events leading to the recruitment of inflammatory cells (neutrophils and macrophages). Although the main function of inflammation is the resolution of infection, several viruses, including the hepatitis C viruses (HCV) have evolved to utilize this host response and make the cellular environments conducive to infection. In majority of infected individuals, HCV causes persistent chronic liver inflammation leading to development of liver cirrhosis and hepatocellular carcinoma. HCV induces reactive oxygen species (ROS) and activates nuclear factor-κB (NF-κB) leading to the activation of cyclooxygenase-2 (Cox-2) that ultimately produces prostaglandin-E2 (PGE2), thus enhancing inflammatory process. Interestingly, HCV further activates NACHT, LRR, and PYD domains-containing protein 3 (NLRP3) inflammasome (a multiprotein complex) by recruiting adaptor protein apoptosis-associated speck-like protein containing a carboxy-terminal CARD (ASC) which are involved in activation of caspase-1 leading to production of interleukin-1beta (IL-1β) and interleukin-18 (IL-18). In this chapter we have highlighted the recent advancements in HCV-induced inflammatory responses and discussed potential future directions to understand the role of inflammation during HCV infection.

Keywords: PAMP, DAMP, TLR, NLRP3, AIM2, RIG-I, IFI16, inflammation, inflammasome, IL-Iβ, Caspase-1, HCV, HBV, herpesvirus

1. Introduction

Inflammation, often triggered by harmful stimuli such as tissue injury and pathogenic infections, is an adaptive response that underlies a wide variety of both physiological and

pathological processes [1]. Inflammation can be acute or chronic. Acute inflammation is generally induced by tissue injury, noxious compounds or invasion of pathogens with general clinical signs like swelling, redness, pain and heat at the site of the insult. Acute inflammation is the initial response of body during which, the small immune-mediating molecules called anaphylatoxins are recruited to site where it stimulates mast cells to release histamine, serotonin and prostaglandins. This event is followed by vasodilation to allow immune cells such as the neutrophils to rush to the site to respond to the causative agent. During the acute stage, the inflammation remains a beneficial process to heal and provide relief within few days. Chronic inflammation, however, lasts for weeks, months or even years and cause tissue damage. At the chronic stage, the inflammation becomes a problem rather than solution to infection or disease. In contrast to acute inflammation, the chronic inflammation is generally seen in viral infections and other hypersensitive disorders where the inflammation is persistent for a longer duration. During chronic inflammation, the primary immune cells are macrophages and T lymphocytes which play crucial roles by producing cytokines and other enzymes that are detrimental to cells. Several studies have focused on the chronic inflammation that occurs during type-2 diabetes, cardiovascular and autoimmune diseases and during localized chronic inflammation that occurs due to chronic infections. In spite of so much advancements made in inflammation biology, the causes and mechanistic details are still partly understood and need an in-depth analysis to completely unravel the mystery.

During pathogenic invasion, the host immune system initiates an immediate defense mechanism. The pathogens are recognized by the pattern-recognition receptors (PRR) [2] that identify pathogen-associated molecular patterns (PAMPs) [3] and danger-associated molecular patterns (DAMPs) to rapidly activate the innate arm of the host immune system, including the secretion of chemokines and cytokines [4]. The PRRs, like the Toll-like receptors (TLRs) [5] are present on the plasma membrane and in the endosomes while the RIG-I-like receptors (RLRs) [6], NOD-like receptors (NLRs) [7] and AIM2-like receptors (ALRs) [8] reside in the cytoplasm. During viral infections, the viral RNA is sensed by TLR3, TLR7 and TLR8, and viral DNA is sensed by TLR9. Similarly, viruses are also recognized by soluble sensors such as the RNA-sensing RIG-like helicases (RIG-I and MDA5) or the DNA-sensing PRRs (DAI and AIM2). The viral RNA in cytoplasm is detected by the helicase domain of either RIG-I or MDA5 followed by the exposure of the caspase recruitment domain (CARD) to interact with the N-terminal of mitochondrial adaptor protein (MAVS). This CARD-CARD interaction leads to dimerization of MAVS in the mitochondria to form the MAVS signalosome which further activates the NF-κB, production of type I interferons (IFNs) and the secretion of proinflammatory cytokines (IL-1β and IL-18) and chemokines [9, 10]. The maturation of IL-1β and IL-18 depends on the proteolytic cleavage of the pro-form of caspase-1 to release the active forms of IL-1β and IL-18 [11]. The formation of the active caspase-1 (p10/p20) is often regulated by multi-protein complexes called the inflammasomes [12].

Several distinct inflammasomes including the NLRP3 inflammasome, the absent in melanoma 2 (AIM2) inflammasome, the γ-interferon-inducible protein 16 (IFI16) inflammasome and the RIG-I inflammasomes have been identified to be activated during specific viral and bacterial infections [13]. Several viruses such as vaccinia virus (VACV) [14], HCV [15], hepatitis B virus (HBV) [16], human papillomavirus [17], mouse cytomegaloviruses (mCMV) [14, 16], influenza

virus [9, 18] and Vesicular stomatitis viruses (VSV) [19] have been reported to activate inflammasomes. In this book chapter, we have reviewed the role of inflammation and discussed the detailed mechanism of activation, following viral invasions, specifically during HCV infection.

2. Overview of inflammatory response to viral infections

2.1. Virus-induced inflammatory response

Inflammation is very crucial in maintaining the homeostasis that's altered during any exogenous stimuli such as the tissue injury or a pathogenic infection. Several viruses are known to induce inflammatory response. The virus is sensed by TLRs (TLR3/7, TLR8/9), RLRs (RIG-I and MDA5) and RNA-dependent protein kinases (PKR), to induce the production of inflammatory mediators and IFNs. The dsRNA is usually sensed through RIG-I and/or TLR3 in the monocytes, macrophages and non-immune cells (endothelial cells, epithelial cells and hepatocytes) whereas in plasmacytoid dendritic cells, TLR7 is highly expressed and acts as the major ssRNA sensor [20–23]. The activation of RLRs and TLRs then promote the secretion of IFNs and proinflammatory cytokines. The inflammation is further amplified when the proinflammatory cytokines and chemokines, such as IL-6, IL-8, tumor necrosis factor alpha (TNF-α) and Rantes starts recruiting other cell types to the infected tissue. These events not only contribute in the control of virus replication but also significantly enhance the inflammatory responses and disease severity.

The endoplasmic reticulum is the major site for protein synthesis including viral protein synthesis that disturbs the ER homeostasis and causes ER stress [24]. The main stress response pathway in the ER is the unfolded protein response (UPR) which has been linked to enhanced cytokine (TNF-α and IL-6) production due to activation of NF-κB and pro-inflammatory transcription factors [25, 26]. Thus the UPR pathway serves as the internal danger signal and compliments the cellular viral sensors to boost subsequent antiviral response [27]. Since the ER stress in the absence of any viral infection also leads to production of IL-1β secretion and cell death, it would be interesting to investigate further if there is a crosstalk between the UPR pathway and inflammasome activation during viral infection. The mitochondrial stress has also been associated with formation of ROS that can result in the activation of NF-κB, Cox-2, PGE2, IL-6 and activating protein-1 (AP-1), that subsequently up-regulate antioxidants and inflammatory pathways, including the ISGs [28].

Several viruses such as influenza viruses (human H1N1 and avian H5N1) have been shown to infect the microglia, astrocytes and neuronal cell lines and produce pro-inflammatory cytokines, ultimately leading to cell apoptosis [29]. A recent study also showed that influenza virus infection of mouse primary cortical neurons enhanced the mRNA levels of inflammatory cytokines, chemokines, and type I IFNs [30]. The Epstein–Barr virus (EBV) also triggers the TNF-α signaling by its LMP1 protein, activating NF-κB and resulting in production of IL-6 and subsequently a number of pro-inflammatory and immune stimulatory cytokines [31–33]. Similarly, the KSHV encodes several genes specially the viral Fas-associated death domain-like IL-1-converting enzyme inhibitory protein (vFLIP) that induce NF-κB activation that subsequently upregulates the chemokine CCL20 and its receptor CCL6. The CCL20 then recruits dendritic

cell and lymphocyte and thus contributes to the inflammatory infiltrate in the Kaposi's sarcoma lesions [34, 35]. In case of hepatitis B and C viruses, the liver cancer develops due to years of inflammation, oxidative stress (OS) and cell death leading to chronic liver damage. The liver infiltrating lymphocytes contributes majorly in the production of pro-inflammatory cytokines such as TNF-α, IL-6 and IL-1β during chronic HBV/HCV infection [36, 37].

2.2. Virus-induced inflammasomes

Several viruses like the influenza viruses, Respiratory syncytial virus (RSV), hepatitis B and C viruses, Dengue virus and herpesviruses have been reported to induce inflammation and activate the inflammasomes (**Table 1**). Few viruses are cleared, while a majority of viruses that cause chronic infection and cancer tend to utilize the inflammasome complex and the cellular milieu for their survival and have successful infection. The various inflammasomes that gets activated during different viral invasions are shown in **Table 1** and **Figure 1**.

The inflammasomes further contribute in secretion of inflammatory cytokines during viral infections. The following inflammasomes have been widely discussed during viral infections:

2.2.1. NLRP3 inflammasome

The NLRP3 inflammasome is the best-studied inflammasome and is known to be activated by viruses belonging to different families, suggesting a common pathway for detection of viruses and appropriate response by the host cells. NLRP3 is a multi-domain protein comprising of the N-terminal caspase recruitment domain (CARD), a PYD, a central nucleotide-binding and oligomerization domain (NACHT) (also termed NOD) and the C-terminal leucine-rich repeats (LRRs) [50]. The N-terminal domain helps in signal transduction by interacting with other CARD or PYD-containing proteins. The central NACHT domain serves as the scaffold protein and helps in oligomerization, thus activating the inflammasome. The LRRs are believed to act as ligand sensors. The formation of NLRP3 inflammasome induces the activation of caspase-1 and production of mature IL-1β and IL-18 [11]. NLRP3 inflammasome has been shown to be activated by ATP mediated efflux of PAMPs [51], lysosome/cathepsin B [52] and Ca^{2+}/ROS [53]. Viruses from different families are known to activate and modulate NLRP3 inflammasomes.

PRR	Pathogens	PAMPs recognized	Cytokines expression modulated	Refs
NLRP3	Influenza virus, Sendai virus, Vaccinia virus, HCV, RSV, VSV and Rabies virus	RNA	IL-1β and IL-18	[15, 18, 38–43]
AIM2	VACV, HBV, HPV and mCMV	Cytoplasmic DNA	IL-1β and IL-18	[14, 16, 17, 44]
RIG-I	Influenza virus, HCV, Rabies virus, JEV, RSV	RNA	Type I IFNs, IL-1β and IL-18	[6, 9, 45, 46]
IFI16	KSHV, EBV, HSV-1	Nuclear DNA	Type I IFNs, IL-1β	[47–49]

Table 1. Virus-induced inflammasome activation and modulation of cytokines.

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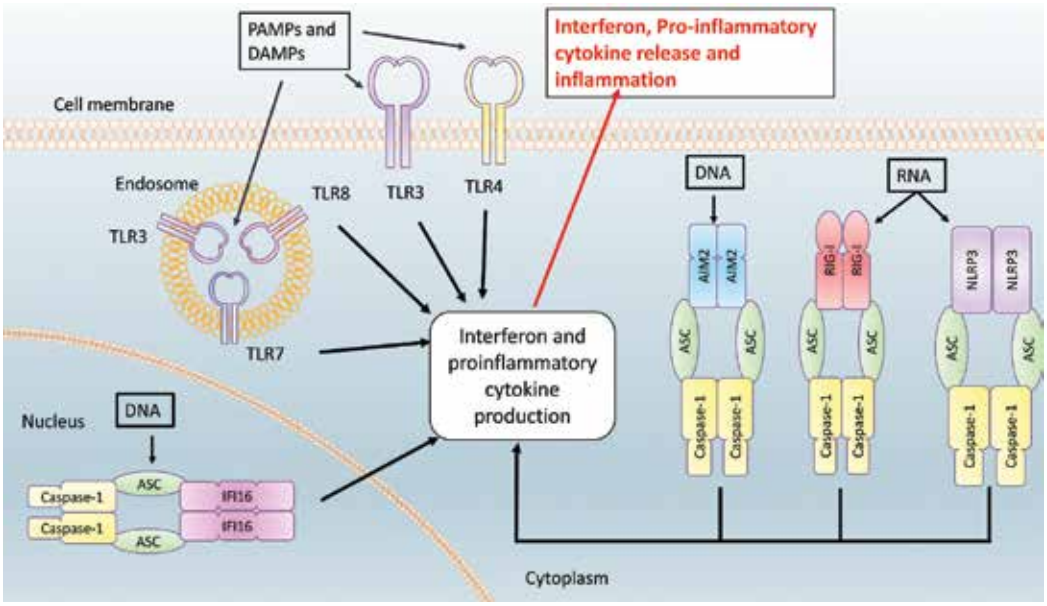

Figure 1. Inflammasome activation during viral infection. Infection with viruses leads to inflammasome activation. Depending on the type of nucleic acid composition of the invading pathogen different types of inflammasomes are activated. TLRs do not form inflammasome but do sense PAMPs and DAMPs associated with pathogens and its associated products. TLRs are located on either the cell membrane (TLR3 and TLR4) or endosome (TLR7 and TLR8). Sensing of PAMPs and DAMPs by TLRs activates cellular pathways which leads to the production of IFNs and proinflammatory cytokines. IFI16 detects DNA in the nucleus and is activated through formation of a complex formed with ASC and caspase-1. Similarly, AIM2 also detects pathogen DNA in the cytoplasm and forms an inflammasome with ASC and caspase-1. Whereas, RIG-I and NLRP3 both sense RNA PAMPs from pathogens, and similar to IFI16 and AIM2, form an inflammasome complex with adaptor ASC and effector caspase-1. Formation of inflammasome complex leads to its activation and release of IFN and proinflammatory cytokines which ultimately causes inflammation.

Influenza viruses are the most common activators of NLRP3 inflammasome [38]. Studies have further shown that the influenza virus proton-specific ion channel M2 protein activates NLRP3 inflammasome in the acidic trans-Golgi network [54]. The hepatitis C virus (JFH-1) also activates the NLRP3 inflammasome in Huh7.5 cells and THP-1 macrophages and leads to the production of IL-1β [15, 43]. The ROS inhibitor diphenyleneiodonium (DPI) has been shown to inhibit the HCV-induced IL-1β production [43]. Thus HCV has been shown to activate the NLRP3 inflammasomes both through the HCV genomic RNA and ROS model. Others viruses like the Rabies virus [42], modified vaccinia virus [14], Japanese encephalitis virus [55] and Rift Valley fever viruses [56] are also shown to induces IL-1β production and NLRP3 inflammasome activation.

Apart from RNA viruses, the DNA viruses are also reported to activate NLRP3 inflammasome. The Herpes simplex virus 1 (HSV-1) infection triggers the association of ASC with NLRP3 along with the production of mature caspase-1 and IL-1β in the human foreskin fibroblasts [49]. Adenovirus activates IL-1β secretion in monocytic cells. The transfected adenoviral DNA was known to activate the inflammasome which was NLRP3 independent, however later in a study, it was observed that adenoviral infection could activate the NLRP3 inflammasome, thus suggesting that NLRP3 inflammasome activation could be dependent on the

route of viral DNA. The study further showed that NLRP3 knockout mice showed decreased IL-1β induction in response to adenoviral infection thus indicating the possibility of other sensors identifying transfected adenoviral DNA in previous studies [57]. In another study, the Varicella-Zoster Virus (VZV) was also demonstrated to activate the NLRP3 followed by recruitment of ASC and caspase-1 in monocytic and melanoma cell lines and in skin xenografts [58]. Few studies have shown the relation of NLRP3 in HBV infections, however the results does not directly correlate the increased expression of NLRP3 in CHB patients with HBV-DNA copy number. Hence the increase in NLRP3 may be due to an indirect effect of HBV such as the liver damage [59]. Another recent study has shown that HBV-HBeAg suppressed the LPS-induced activation of the NLRP3 inflammasome and production of IL-1β by suppressing the NF-κB pathway and ROS production [60]. Since studies about the activation of NLRP3 during HBV infection are still progressing, it would be interesting to understand how HBV modulates inflammasomes for its propagation.

2.2.2. RIG-I inflammasome

The RIG-I, a member of the RLR family, contains two N-terminal CARDs that recruits several adaptor proteins, a central RNA helicase domain that has an ATPase activity and a C-terminal regulatory domain (CTD) that binds to the dsRNA to collectively induce the type I IFN production [61]. The RIG-I has been shown to recognize the dsRNA replication intermediates of several RNA viruses [62]. Influenza virus, HCV, Sendai virus, New castle disease virus, rabies virus and RSV showed defective IFN production in the absence of RIG-I [6]. The role of RIG-I as inflammasome activator has been shown in a study that was conducted with rhabdovirus VSV infection in murine dendritic cells in which there was RIG-I dependent production of IL-1β and IL-18 via NF-κB, caspase-1, and caspase-3 activation. The knockdown of RIG-I in mice inhibited the secretion of IL-1β [19]. Another study however showed conflicting results in which the infection with VSV was shown to be activated by NLRP3 and not by RIG-I [41]. These contrary results highlight the possible dual role of RIG-I in the inflammasome and type 1 IFN pathways. A study conducted with influenza virus infection in the primary human bronchial epithelial cells demonstrated both RIG-I-dependent priming of the NLRP3 inflammasome as well as direct RIG-I-mediated inflammasome activation [9]. Thus extensive research is still needed to analyze the roles of RIG-I during viral infections.

2.2.3. AIM2 inflammasome

The AIM2 is a member of the interferon (IFN)-inducible protein with a 200 amino acid repeat family (also known as the HIN200 family of IFI200 family) containing an N-terminal PYD and a C-terminal HIN200 domain. The family includes at least six members in mice (IFI202, IFI203, IFI204, IFI205, PYHIN1 and AIM2) and four members in humans (IFI16, MNDA, IFIX and AIM2). Studies have demonstrated that AIM2 senses the cytoplasmic bacterial, viral, or even the host double-stranded DNA (dsDNA) [8, 16]. The AIM2 utilizes its PYD domain to interact with ASC and recruit caspase-1 for the AIM2 inflammasome formation and IL-1β and IL-18 secretion [16]. AIM2 has been shown to be required for activation of caspase-1 during the VACV and MCMV infection in cell culture system but not during the HSV-1 infection [14, 63]. The sensing of VACV

and MCMV but not HSV-1 indicates that few viruses have evolved to block the AIM2 mediated recognition of their genome and downstream signaling. It has been further shown that AIM2$^{-/-}$ mice infected with MCMV were defective in IL-18 and IFN-γ production as compared to their control littermates [14]. The human hepatocytes have also been shown to express AIM2. An *in vitro* study has shown that the AIM2 senses the hepatitis B virus in hepatocytes and increases the production of IL-18. Further, the study showed that the expression of AIM2 in chronic hepatitis B (CHB) patients was higher than that of controls and which positively correlated to the severity of liver inflammation [64]. In another study conducted on peripheral blood mononuclear cells (PBMCs) from patients with acute hepatitis B (AHB) and CHB during different clinical phases, the expression of AIM2, IL-1β, and IL-18 was observed to be significantly high in AHB compared with expression in CHB patient samples [44]. The low expression in CHB patients also suggests that AIM2 may be associated with the chronic development of hepatitis [44]. It would be interesting to study if all the family of DNA viruses is sensed by the AIM2 inflammasomes.

2.2.4. IFI16 inflammasome

Similar to AIM2, the IFI16 belongs to the ALR family however they differ in their cellular localization. The former is strictly cytosolic while the latter is mainly localized in the nucleus due to its nuclear localizing sequence (NLS). Since both AIM2 and IFI16 recognizes DNA, these sensors are also reported to get activated by self-DNA, potentially leading to various autoimmune and auto inflammatory diseases such as lupus pathogenesis [65], Sjögren's syndrome [66] and systemic sclerosis [67]. The IFI16 is also known to sense viral DNA during infection. A study conducted on KSHV has shown that IFI16 recognized the viral DNA in the nucleus and later translocated to cytoplasm only in infected cells [68]. Upon recognition of the KSHV genome, the IFI16 is acetylated in the nucleus and later redistributed to the cytoplasm with the help of BRCA1 [48, 69]. Among others, the herpes simplex virus 1 (HSV-1), Epstein–Barr virus (EBV), and bovine herpesvirus 1 (BoHV-1) are also reported to activate the IFI16-ASC inflammasomes and produce inflammatory cytokine IL-1β [47, 49, 70].

3. Hepatitis C virus and liver inflammation

Hepatitis C virus is a hepatotropic virus, belongs to the *Flaviviridae* family. It is a positive sense single-stranded RNA virus. The RNA genome is present in an icosahedral structure made up of core proteins, which is further encapsulated in lipid bilayer which contains E1/E2 glycoproteins in a heterodimer on the membrane [71]. The RNA genome contains a 5'UTR which has an internal ribosomal entry site (IRES) and is required for cap–independent translation [72, 73]. On the other hand, the 3'UTR consists of mainly a poly (U/UC) tract and X-tail which have been shown to be required for replication of viral RNA [74, 75]. In between the two UTRs exists the genomic region which translates into a 3000aa polyprotein which is cleaved by host peptidases and viral proteins to form structural (core, E1 and E2) proteins, p7 and non-structural (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) proteins. The virus is known to cause chronic infections in liver and eventually cancer (**Figure 2**). HCV causes chronic inflammation leading to liver fibrosis, steatosis, cirrhosis and finally hepatocellular carcinoma (HCC).

Inflammation is a crucial physiological event that occurs during chronic HCV infection. Chronic inflammation is defined by the persistence of inflammatory cells and destruction of liver cells. The liver cells have a unique regenerative capacity and can replace a significant loss of liver cells by compensatory proliferation. However, the chronic liver damage and regeneration results in scarring of liver called liver fibrosis. The fibrotic stage is characterized by the activation of HSCs and extracellular matrix (ECM) secretion. The liver fibrosis is also enhanced due to promotion of activated hepatic stellate cells (HSCs) survival in a NF-κB dependent manner by the KCs and recruited macrophages [76]. The ROS released by KCs and NADPH oxidase stimulated ROS production in HSCs and hepatocytes, result in robust induction of OS leading to DNA damage, enhanced expression of proinflammatory genes, fibrogenesis and malignancy [77]. The fibrotic stage gradually progresses to late stage of fibrosis called cirrhosis, which is the hallmark of an irreversible advanced stage liver injury. At this stage the dense bands of fibrotic scar develops into abnormal nodules of hepatocytes, resulting mainly from regenerative hyperplasia, separated by fibrous tissues. The disease progression eventually leads to the loss of normal functionality of liver such as xenobiotic metabolism and the metabolism of carbohydrates, proteins and other crucial molecules. In case of HCV infection, the complication progresses as a mild liver disease for 15–20 years after which a substantial number of individuals develop liver cirrhosis with clinical complications such as ascites, variceal hemorrhage and hepatic encephalopathy [78]. The ultimate complication of cirrhosis is the development of hepatocellular carcinoma.

In HCV infected individuals, besides a local inflammation in the liver, a mild systemic inflammation is also observed due to increased pro-inflammatory cytokine serum levels and

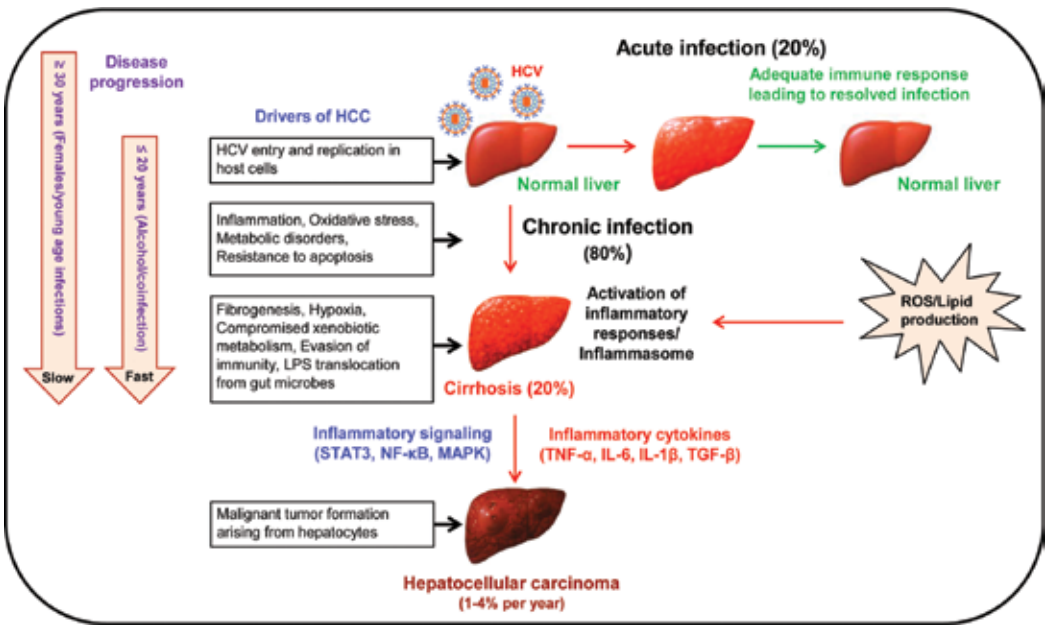

Figure 2. Schematic diagram representing different stages of HCV-induced liver disease progression.

activation of blood monocytes. The OS generated during chronic infection also plays key roles in the development of local and systemic inflammation. HCV proteins activate several pathways responsible for increased inflammatory response. The NS5A, for example, promotes upregulation of Cox-2 which contributes to chronic inflammation and fibrosis through production of various prostaglandins [79]. The chronic liver damage due to continuous inflammatory response (various inflammatory cytokines) and OS for several years ultimately leads to liver cancer [36]. Similarly, HCV infection also leads to an enrichment of proinflammatory cytokines in the liver cells ultimately leading to increased secretion of TNF-α, IL-6 and IL-1β [80]. These inflammatory events make the HSCs highly responsive to the transforming growth factor β (TGF-β) [81] that promotes hepatic fibrogenesis and eventually the progression and prognosis of HCC [82, 83]. HCV also induces the ER stress that increases the intracellular ROS levels which ultimately leads to increase in inflammatory gene expression by activation of NF-κB, AP-1 and STAT3 [84, 85]. A study has shown that the HCV core induces lipid accumulation leading to increased ROS production and inflammation ultimately promoting the HCC in transgenic mice [86, 87]. Osteopontin (OPN) is a cytokine that either remain intracellular or is secreted to allow both autocrine and paracrine signaling. Studies have shown the correlation of hepatic inflammation with increased expression of OPN [88, 89]. Recent studies have also shown that OPN is a crucial player during HCV infection and plays roles in epithelial to mesenchymal transition of hepatocytes [90, 91].

3.1. Role of various cytokines in HCV-induced inflammation

Cytokines belong to a large group of proteins that are secreted from specific cells of the immune system and perform a wide range of biological functions including innate and acquired immunity, hematopoiesis and inflammation. They mainly include the interleukins, chemokines, IFNs, TNF etc. Viral proteins and dsRNA from HCV triggers the induction of proinflammatory cytokines and chemokines. HCV core protein has been shown to induce inflammatory cytokines through the STAT3 signaling pathway [92]. A study further showed that a cross-talk existed between the HSCs and HCV-infected hepatocytes. The IL-1β secreted by HSCs co-cultured with the hepatocytes, ignited the production of several pro-inflammatory cytokines and chemokines, such as IL-6, IL-8, MIP-1α and MIP-1β, by the hepatocytes [93]. The HCV proteins (NS3, NS4 and NS5) are also reported to induce the human Kupffer cells (KCs) to synthesize inflammatory cytokines such as TNF-α and IL-1β [94]. The HCV-NS5A protein has been shown to induce high levels of pro-inflammatory chemokine IL-8 to inhibit IFN-α thus facilitating the viral replication despite IFN α/β induction [95]. *In vitro* studies have shown that IL-10 production is regulated by HCV structural proteins to inhibit IL-12 production in myeloid cells. This also correlated with reduced IL-12 levels observed in chronic hepatitis C patients [96]. Serum cytokine levels were evaluated in HCV patients, and it was observed that both T helper (Th) 1 and Th2 lymphocytes were highly associated with chronic HCV infection [97]. This lead to the increased production of IL-2, IL-4, and IL-6 cytokines in all chronic active hepatitis patients [97]. Liver fibrosis has been shown to progress due to the persistent inflammation activating the HSCs, myofibroblasts, and fibroblasts which are regulated by pro-inflammatory cytokines such as TGF-β, IL-6, TNF-α, CCL21, and platelet-derived growth factor (PDGF) [98]. The HCV related mixed cryoglobulinemia

(MC) (MC + HCV) is an extrahepatic disease associated with HCV infection. In a study, the MC + HCV was shown to express significantly higher mean IL-1β, IL-6, and TNF-α levels than the controls or the HCV patients [99]. A recent study has shown the importance of Th17/IL-17 axis in HCV-induced chronic hepatitis and progression to cirrhosis. It promotes the recruitment of inflammatory cells and cytokines IL-6 and IL-23. A similar observation was also made in HCV patients with orthotopic liver transplantation (OLT). The recipients with HCV-induced allograft fibrosis or cirrhosis presented with higher levels of HCV-specific Th17 cells along with proinflammatory mediators (IL-17, IL-1β, IL-6, IL-8, and MCP-1) [100]. In a study conducted to analyze the expression of cytokines in HCV infected patients, it was observed that TNF-α expression was localized mainly in liver sinusoidal cells (macrophages, endothelial cells) and a high proportion of hepatocytes demonstrated expression of TNF-α, IL-1α, and IL-2 [101]. IL-32 has also been shown to be expressed by human hepatocytes and hepatoma cells and is involved in HCV-associated liver inflammation [102]. In addition, IL-32 was found to be constitutively expressed in the human hepatoma cells and was observed to be upregulated by IL-1β and TNF-α [102].

3.2. HCV-induced oxidative stress adds to inflammatory response

Oxidative stress plays a significant role in HCV-induced liver damage. HCV infection has also been reported to activate the liver-residing macrophages- Kupffer cells (KC) and result in ROS production. The activated KCs enhance the production of TNF-α and ROS as a mechanism to cope with HCV infection by killing hepatocytes [103]. HCV has also been shown to induce OS through calcium signaling [84, 104, 105]. The HCV infection also induces ROS that stimulates the NF-κB to activate Cox-2. This event ultimately leads to overexpression of Cox-2 thereby increasing the levels of pro-inflammatory molecules, PGE_2 (**Figure 3**) [104]. The ROS also activates a transcription factor, STAT-3, that controls important cellular processes required for cell survival, proliferation, differentiation and oncogenesis [106] and constitutive activation of NF-κB and STAT-3 by HCV has been shown to be involved in acute and chronic liver disease associated with HCV infection [107]. ROS has also been shown to increase the proliferation of HSCs as well as TGF-β and collagen synthesis to promote fibrogenesis [108]. Hepatic steatosis, reported in more than 50% of HCV-infected patients, has also been linked to OS in CHC patients infected with HCV genotype non-3 [109]. The HCV-infected human hepatoma cells enhance the expression of TGF-β1 by induction of transcription factors AP-1, Sp1, NF-κB and STAT-3 via OS [110].

3.3. Role of inflammasomes in HCV-induced inflammatory response

HCV infection in liver cells stimulates host responses which triggers PRRs to recognize HCV components. Recognition usually occurs through TLR3 and TLR7 on either the cell surface or the endosomal compartments during HCV infection (**Figure 3**) [111]. TLR expression and recognition of HCV associated PAMPs has led to production of IFN as well as activation of NF-κB mediated inflammatory molecules which ultimately cause inflammation. TLR3 signaling pathway is led by TIR-domain-containing adaptor-inducing interferon-B (TRIF) which activates IRF-3 and NF-κB which produces pro-inflammatory cytokines, chemokines and type I IFN. Even though TLR3 expression was observed in HCV infected cells it was identified that the downstream signaling is impaired by HCV non-structural proteins NS3/4A, NS5A

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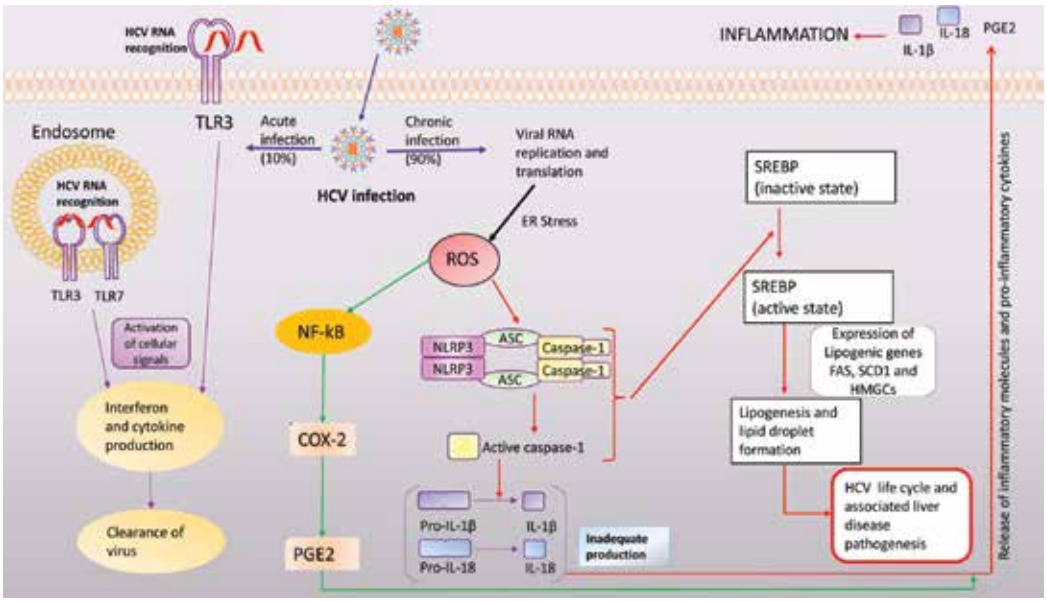

Figure 3. HCV-induced inflammasome regulates liver disease pathogenesis.

and NS5B [112] and also by decreasing the expression of TLR3 adaptor TRIF [113]. TLR7 activation leads to formation of a complex with MyD88, TRAF6, IRAK4 and IRAK1, which further activates IRF7 and induces interferon signaling.

During HCV infection HCV PAMPs are not only recognized by TLRs but also by RIG-I. It has been observed that HCV dsRNA is recognized by RIG-I during initial hours of HCV viral infection [114]. dsRNA binding to RIG-I initiates an interaction between 14-3-3ε and E3-ubiquitin ligase TRIM25 [115, 116]. This interaction leads to another interaction of RIG-I with MAVS, which contributes to IRF3 and NF-κB signalosome activation and production of IFNs [117, 118]. It was identified by Baril et al. that HCV prevents further signal transduction of RIG-I through proteolytic cleavage of MAVS by HCV NS3/NS4A protease [119]. MAVS cleavage results in disruption of RIG-I mediated IFN production during HCV infection [120].

HCV has also been shown to activate NLRP3 inflammasome in infected liver cells. A study has shown that HCV increases NLRP3 expression in liver [121]. In another study Burdette et al. for the first time showed induction and assembly of NLRP3 inflammasome in human hepatoma cells infected with HCV (JFH-1) (**Figure 3**) [15]. The study demonstrated that NLRP3, upon sensing the HCV, recruits an adaptor protein ASC for the assembly of the inflammasome complex. The study also highlighted that the activation of IL-1β in HCV infected cells was achieved by proteolytic processing of pro-caspase-1 into mature caspase-1 [15] and siRNA mediated cleavage of NALP3, ASC and caspase-1 abrogated the IL-1β secretion suggesting that HCV infected hepatoma cells (epithelial) activates NLRP3 inflammasome [15]. In another study by Boaru et al., it was shown that NLRP3 inflammasome was prominently assembled in liver sinusoidal endothelial cells and KCs, moderately in cultured HSCs and periportal myofibroblasts and almost absent in primary hepatocytes [122]. Studies have also shown that NLRP3 inflammasome was

not activated in human hepatoma cells or primary hepatocytes [43, 123]. The possible reason for not observing the inflammasome in primary hepatocytes could be explained by the fact that the authors relied on the detection of mature IL-1β and IL-18. There are other studies that support that hepatocytes express and also activates the inflammasome complex, however do not secrete detectable amounts of IL-1β and IL-18 as compared to immune cells [124, 125]. This also suggests that the activation of inflammasome in epithelial cells might be performing cytokine independent functions. Negash et al. also showed that KCs were the major IL-1β-producing cell population during HCV infection and that the serum levels of IL-1β were significantly increased in patients with CHC [43]. They also showed that exposure of THP1 cells to HCV-induced IL-1β production and secretion via NLRP3 inflammasome pathway. All these events lead to enhanced proinflammatory cytokine and immune-regulatory gene expression [43]. In another study, Chen et al. reported that HCV-induced ROS production activated the NLRP3 inflammasome and subsequent IL-1β secretion [40]. Similarly, Shrivastava et al. also showed that the inflammatory cytokines IL-1β and IL-18 were produced through the activation of NF-κB pathway and induction of ROS. In THP-1 cells they observed that the production of these cytokines was through the NLRP3 inflammasome activation and caspase-1 cleavage [123]. Interestingly, caspase-1 activation has been shown to not only result in pro-inflammatory cytokine production but also regulation of many other cellular pathways. A study by Li et al. identified 40 genes regulated by caspase-1 in various tissues [126]. Previously, Grucel et al. showed caspase-1 induced activation of sterol regulatory element binding proteins (SREBP) in response to bacterial pore forming toxins. Thus, the contradicting results observed for the NLRP3 inflammasome activation in human hepatocytes cells and immune cells could be due to the possibility that activation of the NLRP3 inflammasome leads to regulation of other cellular genes or pathways other than production of pro-inflammatory cytokines. Therefore, the recent study from our lab has shown that HCV exploits the NLRP3 inflammasome to activate the SREBPs and host lipid metabolism for liver disease pathogenesis (**Figure 3**) [39]. In addition, IFN has been shown to inhibit NLRP3 inflammasome by blocking the caspase-1 dependent IL-1β maturation [127]. Thus therapeutically targeting NLRP3 inflammasome complex or IL-1β could provide better interventions in managing liver inflammation in CHC patients.

4. Therapeutic approaches to manage HCV-induced inflammation

HCV has been linked to several other diseases including the lymphoproliferative diseases [128], cardiovascular diseases [129], and atherosclerosis [130], and neuropsychiatric symptoms [131]. Since inflammation plays a key role in disease progression in chronic hepatitis C patients, a therapeutic method to anti-inflammatory approach would result in better management of the disease. Chen et al. have shown the beneficial effect of the aqueous extract of an edible seaweed *Gracilaria tenuistipitata* in inhibition of HCV replication by suppressing the Cox-2 protein and thus reducing inflammatory response [132]. Sorafenib is a chemotherapeutic agent that has been shown to inhibit the Raf/ERK pro-inflammatory and pro-fibrotic signaling pathways [133]. Similarly animal model have been used to show the effect of TNFα inhibitors on reduction of IL-6 and TGF-β [134], however the efficacy of such anti-inflammatory drugs will need extensive research owing to the risk of interference with the IFN therapy prescribed for HCV

Drugs	Disease	Role	Refs
Pre-existing treatments			
Sorafenib	Hepatocellular carcinoma	Inhibits Raf/ERK	[130]
Corticosteroids	Liver disorders	Anti-inflammatory	[138]
Cyclosporine	Autoimmune hepatitis	Calcineurin inhibitor, reduces cytokines, inhibits TGF-β and IL-4	[139]
Azathioprine	Autoimmune hepatitis	Anti-inflammatory	[140]
Budesonide	Autoimmune hepatitis	Anti-inflammatory synthetic corticosteroid	[141]
Tacrolimus	Autoimmune hepatitis	Calcineurin inhibitor	[142]
Emerging or possible treatments for liver inflammation			
Cenicriviroc	Non-alcoholic steatohepatitis (NASH) and liver fibrosis	Inhibits chemokine receptors CCR2/ CCR5	[143]
Fresolimumab	Systemic sclerosis	Neutralizes TGF-β	[144]
Pioglitazone	Hepatic steatosis due to HIV/ HCV infections	Acts as a PPARγ agonist, helps in reduction of ROS	[145]
Glycyrrhizin	Chronic hepatitis C and F2/F3 liver fibrosis	Anti-oxidant	[145]
Resveratrol	Non-alcoholic steatohepatitis (NASH)	Anti-oxidant	[146]
Humira	Certain arthritis such as rheumatoid and psoriatic	TNF-α blockers	[147]
Celecoxib	Pain and inflammation	Cox-2 inhibitor	[148]
Canakinumab	Acute and chronic non-infectious inflammatory diseases	IL-1β inhibitor	[135]
Pentoxifylline	Liver fibrosis, Non-alcoholic steatohepatitis (NASH), Primary biliary cirrhosis (PBC), Alcoholic liver disease	TNFα suppressing phosphodiesterase inhibitor	[136, 137]
Ursodeoxycholic acid	Primary biliary cirrhosis (PBC), Autoimmune hepatitis	Decreases TGF-β signaling and oxidative stress, TNF-α, IL-1α, IL-1β, and IL-6, IL-10 NF-κB	[149, 150]

Table 2. Pre-existing and emerging or possible treatments used against hepatic inflammation observed in various liver diseases.

mediated hepatitis. Microbial translocation in HCV infected resident KCs could also serve as a good platform to minimize the LPS-induced inflammasome response [135]. Dammacco et al. in their study showed that triple therapy with pegylated IFN-α, ribavirin, and rituximab (RTX) to patients with HCV-related cryoglobulinemia gave significantly better results than those who only got pegylated IFN-α and ribavirin [136]. Since IL-1β is directly involved in inflammatory response, and hence Canakinumab, a human monoclonal antibody that selectively inhibits IL-1β was shown to inhibit many inflammatory biomarkers [137].

Pentoxifylline (PTX) is a methylxanthine derivative with a variety of anti-inflammatory and antifibrotic effects, has been shown to be effective in liver diseases like the alcoholic liver disease [151], fibrosis/cirrhosis [152]. The drug also decreases the levels of TNF-α, IL-1, IL-6 and TGF-β which holds significant therapeutic potential [153]. There are few preexisting and possible emerging therapies against hepatic inflammation and liver disease available which are listed in **Table 2**.

5. Conclusions

Inflammation is a crucial part of human immune response that kicks into high gear during any tissue injury or invasion of harmful bacteria and viruses. When a cell dies, it stimulates a number of processes including the rapid recruitment of innate immune components from blood to generate an inflammatory response. This is a double-edged sword that in one hand protects and heals the injured tissues while on the other hand cause significant damage and disease progression. Both bacterial and viral infections have been well recognized as potent source of inflammation. Various studies have shown that these pathogens induce inflammation and in some cases the inflammation is continuous for several years ultimately contributing to cancer. With some oncogenic viruses, the unceasing inflammation significantly contributes to tumor formation. Growing evidences support the crucial role of HBV- and HCV-induced inflammatory responses in liver for both the reversal of disease as well as pathogenesis of hepatic and extrahepatic diseases. The persistent HCV infection leads to chronic inflammation which has been shown to be the primary cause of liver fibrosis and cancer. More importantly the epithelial cells mediate the progression from fibrotic to carcinogenic stage. It has been shown that during the chronic HCV infection, the hepatocytes show a transition from pSmad3C pathway, characteristics of mature epithelial cells, to JNK/pSmad3L pathway which favors the liver fibrosis and also increase the risk of cancer. Several studies have shown the roles of inflammatory mediator such as the IL-6, Cox-2, NF-κB and more recently the activation of inflammasomes, as major contributors in HCV pathogenesis. The HCV-induced inflammation still needs more studies to better elucidate the treatment options and to date, the novel therapeutic targets for inflammation, seems to be a good option for better management of disease, especially in non-responders to the standard antiviral treatment.

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Conflict of interest

None.

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References

[1] Nathan C. Points of control in inflammation. Nature. 2002;**420**(6917):846-852

[2] Medzhitov R, Janeway Jr CA. Decoding the patterns of self and nonself by the innate immune system. Science. 2002;**296**(5566):298-300

[3] Janeway Jr CA, Medzhitov R. Innate immune recognition. Annual Review of Immunology. 2002;**20**:197-216

[4] Wilkins C, Gale Jr M. Recognition of viruses by cytoplasmic sensors. Current Opinion in Immunology. 2010;**22**(1):41-47

[5] Kawai T, Akira S. TLR signaling. Seminars in Immunology. 2007;**19**(1):24-32

[6] Kato H, Sato S, Yoneyama M, Yamamoto M, Uematsu S, Matsui K, et al. Cell type-specific involvement of RIG-I in antiviral response. Immunity. 2005;**23**(1):19-28

[7] Ting JP, Lovering RC, Alnemri ES, Bertin J, Boss JM, Davis BK, et al. The NLR gene family: A standard nomenclature. Immunity. 2008;**28**(3):285-287

[8] Roberts TL, Idris A, Dunn JA, Kelly GM, Burnton CM, Hodgson S, et al. HIN-200 proteins regulate caspase activation in response to foreign cytoplasmic DNA. Science. 2009; **323**(5917):1057-1060

[9] Pothlichet J, Meunier I, Davis BK, Ting JP, Skamene E, von Messling V, et al. Type I IFN triggers RIG-I/TLR3/NLRP3-dependent inflammasome activation in influenza A virus infected cells. PLoS Pathogens. 2013;**9**(4):e1003256

[10] Garlanda C, Dinarello CA, Mantovani A. The interleukin-1 family: Back to the future. Immunity. 2013;**39**(6):1003-1018

[11] Martinon F, Tschopp J. Inflammatory caspases and inflammasomes: Master switches of inflammation. Cell Death and Differentiation. 2007;**14**(1):10-22

[12] Martinon F, Burns K, Tschopp J. The inflammasome: A molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. Molecular Cell. 2002; **10**(2):417-426

[13] Brodsky IE, Monack D. NLR-mediated control of inflammasome assembly in the host response against bacterial pathogens. Seminars in Immunology. 2009;**21**(4):199-207

[14] Rathinam VA, Jiang Z, Waggoner SN, Sharma S, Cole LE, Waggoner L, et al. The AIM2 inflammasome is essential for host defense against cytosolic bacteria and DNA viruses. Nature Immunology. 2010;**11**(5):395-402

[15] Burdette D, Haskett A, Presser L, McRae S, Iqbal J, Waris G. Hepatitis C virus activates interleukin-1beta via caspase-1-inflammasome complex. The Journal of General Virology. 2012;**93**(Pt 2):235-246

[16] Hornung V, Ablasser A, Charrel-Dennis M, Bauernfeind F, Horvath G, Caffrey DR, et al. AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. Nature. 2009;**458**(7237):514-518

[17] Reinholz M, Kawakami Y, Salzer S, Kreuter A, Dombrowski Y, Koglin S, et al. HPV16 activates the AIM2 inflammasome in keratinocytes. Archives of Dermatological Research. 2013;**305**(8):723-732

[18] Kuriakose T, Kanneganti TD. Regulation and functions of NLRP3 inflammasome during influenza virus infection. Molecular Immunology. 2017;**86**:56-64

[19] Poeck H, Bscheider M, Gross O, Finger K, Roth S, Rebsamen M, et al. Recognition of RNA virus by RIG-I results in activation of CARD9 and inflammasome signaling for interleukin 1 beta production. Nature Immunology. 2010;**11**(1):63-69

[20] Sun P, Fernandez S, Marovich MA, Palmer DR, Celluzzi CM, Boonnak K, et al. Functional characterization of ex vivo blood myeloid and plasmacytoid dendritic cells after infection with dengue virus. Virology. 2009;**383**(2):207-215

[21] Tsai YT, Chang SY, Lee CN, Kao CL. Human TLR3 recognizes dengue virus and modulates viral replication in vitro. Cellular Microbiology. 2009;**11**(4):604-615

[22] da Conceicao TM, Rust NM, Berbel AC, Martins NB, do Nascimento Santos CA, Da Poian AT, et al. Essential role of RIG-I in the activation of endothelial cells by dengue virus. Virology. 2013;**435**(2):281-292

[23] Nasirudeen AM, Wong HH, Thien P, Xu S, Lam KP, Liu DX. RIG-I, MDA5 and TLR3 synergistically play an important role in restriction of dengue virus infection. PLoS Neglected Tropical Diseases. 2011;**5**(1):e926

[24] Zhang L, Wang A. Virus-induced ER stress and the unfolded protein response. Frontiers in Plant Science. 2012;**3**:293

[25] Carroll TP, Greene CM, O'Connor CA, Nolan AM, O'Neill SJ, McElvaney NG. Evidence for unfolded protein response activation in monocytes from individuals with alpha-1 antitrypsin deficiency. Journal of Immunology. 2010;**184**(8):4538-4546

[26] Eizirik DL, Miani M, Cardozo AK. Signalling danger: Endoplasmic reticulum stress and the unfolded protein response in pancreatic islet inflammation. Diabetologia. 2013; **56**(2):234-241

[27] Smith JA. A new paradigm: Innate immune sensing of viruses via the unfolded protein response. Frontiers in Microbiology. 2014;**5**:222

[28] Schreck R, Rieber P, Baeuerle PA. Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B transcription factor and HIV-1. The EMBO Journal. 1991;**10**(8):2247-2258

[29] Wang G, Zhang J, Li W, Xin G, Su Y, Gao Y, et al. Apoptosis and proinflammatory cytokine responses of primary mouse microglia and astrocytes induced by human H1N1 and avian H5N1 influenza viruses. Cellular & Molecular Immunology. 2008;**5**(2):113-120

[30] Wang G, Li R, Jiang Z, Gu L, Chen Y, Dai J, et al. Influenza virus induces inflammatory response in mouse primary cortical neurons with limited viral replication. BioMed Research International. 2016;**2016**:8076989

[31] Eliopoulos AG, Stack M, Dawson CW, Kaye KM, Hodgkin L, Sihota S, et al. Epstein-Barr virus-encoded LMP1 and CD40 mediate IL-6 production in epithelial cells via an NF-kappaB pathway involving TNF receptor-associated factors. Oncogene. 1997; **14**(24):2899-2916

[32] Mosialos G, Birkenbach M, Yalamanchili R, VanArsdale T, Ware C, Kieff E. The Epstein-Barr virus transforming protein LMP1 engages signaling proteins for the tumor necrosis factor receptor family. Cell. 1995;**80**(3):389-399

[33] Maggio E, van den Berg A, Diepstra A, Kluiver J, Visser L, Poppema S. Chemokines, cytokines and their receptors in Hodgkin's lymphoma cell lines and tissues. Annals of Oncology. 2002;**13**(Suppl 1):52-56

[34] Punj V, Matta H, Schamus S, Yang T, Chang Y, Chaudhary PM. Induction of CCL20 production by Kaposi sarcoma-associated herpesvirus: Role of viral FLICE inhibitory protein K13-induced NF-kappaB activation. Blood. 2009;**113**(22):5660-5668

[35] Ensoli B, Sturzl M. Kaposi's sarcoma: A result of the interplay among inflammatory cytokines, angiogenic factors and viral agents. Cytokine & Growth Factor Reviews. 1998; **9**(1):63-83

[36] Falasca K, Ucciferri C, Dalessandro M, Zingariello P, Mancino P, Petrarca C, et al. Cytokine patterns correlate with liver damage in patients with chronic hepatitis B and C. Annals of Clinical and Laboratory Science. 2006;**36**(2):144-150

[37] Shukla R, Yue J, Siouda M, Gheit T, Hantz O, Merle P, et al. Proinflammatory cytokine TNF-alpha increases the stability of hepatitis B virus X protein through NF-kappaB signaling. Carcinogenesis. 2011;**32**(7):978-985

[38] Allen IC, Scull MA, Moore CB, Holl EK, McElvania-TeKippe E, Taxman DJ, et al. The NLRP3 inflammasome mediates in vivo innate immunity to influenza A virus through recognition of viral RNA. Immunity. 2009;**30**(4):556-565

[39] McRae S, Iqbal J, Sarkar-Dutta M, Lane S, Nagaraj A, Ali N, et al. The hepatitis C virus-induced NLRP3 inflammasome activates the sterol regulatory element-binding protein

(SREBP) and regulates lipid metabolism. The Journal of Biological Chemistry. 2016; **291**(7):3254-3267

[40] Chen W, Xu Y, Li H, Tao W, Xiang Y, Huang B, et al. HCV genomic RNA activates the NLRP3 inflammasome in human myeloid cells. PLoS One. 2014;**9**(1):e84953

[41] Rajan JV, Rodriguez D, Miao EA, Aderem A. The NLRP3 inflammasome detects encephalomyocarditis virus and vesicular stomatitis virus infection. Journal of Virology. 2011; **85**(9):4167-4172

[42] Lawrence TM, Hudacek AW, de Zoete MR, Flavell RA, Schnell MJ. Rabies virus is recognized by the NLRP3 inflammasome and activates interleukin-1beta release in murine dendritic cells. Journal of Virology 2013;**87**(10):5848-5857

[43] Negash AA, Ramos HJ, Crochet N, Lau DT, Doehle B, Papic N, et al. IL-1beta production through the NLRP3 inflammasome by hepatic macrophages links hepatitis C virus infection with liver inflammation and disease. PLoS Pathogens. 2013;**9**(4):e1003330

[44] Wu DL, Xu GH, Lu SM, Ma BL, Miao NZ, Liu XB, et al. Correlation of AIM2 expression in peripheral blood mononuclear cells from humans with acute and chronic hepatitis B. Human Immunology. 2013;**74**(5):514-521

[45] Saito T, Owen DM, Jiang F, Marcotrigiano J, Gale Jr M. Innate immunity induced by composition-dependent RIG-I recognition of hepatitis C virus RNA. Nature. 2008; **454**(7203):523-527

[46] Loo YM, Fornek J, Crochet N, Bajwa G, Perwitasari O, Martinez-Sobrido L, et al. Distinct RIG-I and MDA5 signaling by RNA viruses in innate immunity. Journal of Virology. 2008;**82**(1):335-345

[47] Ansari MA, Singh VV, Dutta S, Veettil MV, Dutta D, Chikoti L, et al. Constitutive interferon-inducible protein 16-inflammasome activation during Epstein-Barr virus latency I, II, and III in B and epithelial cells. Journal of Virology. 2013;**87**(15):8606-8623

[48] Ansari MA, Dutta S, Veettil MV, Dutta D, Iqbal J, Kumar B, et al. Herpesvirus genome recognition induced acetylation of nuclear IFI16 Is essential for its cytoplasmic translocation, inflammasome and IFN-beta responses. PLoS Pathogens. 2015;**11**(7):e1005019

[49] Johnson KE, Chikoti L, Chandran B. Herpes simplex virus 1 infection induces activation and subsequent inhibition of the IFI16 and NLRP3 inflammasomes. Journal of Virology. 2013;**87**(9):5005-5018

[50] Kufer TA, Fritz JH, Philpott DJ. NACHT-LRR proteins (NLRs) in bacterial infection and immunity. Trends in Microbiology. 2005;**13**(8):381-388

[51] He Y, Hara H, Nunez G. Mechanism and regulation of NLRP3 inflammasome activation. Trends in Biochemical Sciences. 2016;**41**(12):1012-1021

[52] Hornung V, Bauernfeind F, Halle A, Samstad EO, Kono H, Rock KL, et al. Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. Nature Immunology. 2008;**9**(8):847-856

[53] Cruz CM, Rinna A, Forman HJ, Ventura AL, Persechini PM, Ojcius DM. ATP activates a reactive oxygen species-dependent oxidative stress response and secretion of proinflammatory cytokines in macrophages. The Journal of Biological Chemistry. 2007;**282**(5):2871-2879

[54] Ichinohe T, Pang IK, Iwasaki A. Influenza virus activates inflammasomes via its intracellular M2 ion channel. Nature Immunology. 2010;**11**(5):404-410

[55] Kaushik DK, Gupta M, Kumawat KL, Basu A. NLRP3 inflammasome: Key mediator of neuroinflammation in murine Japanese encephalitis. PLoS One. 2012;**7**(2):e32270

[56] Ermler ME, Traylor Z, Patel K, Schattgen SA, Vanaja SK, Fitzgerald KA, et al. Rift Valley fever virus infection induces activation of the NLRP3 inflammasome. Virology. 2014;**449**:174-180

[57] Muruve DA, Petrilli V, Zaiss AK, White LR, Clark SA, Ross PJ, et al. The inflammasome recognizes cytosolic microbial and host DNA and triggers an innate immune response. Nature. 2008;**452**(7183):103-107

[58] Nour AM, Reichelt M, Ku CC, Ho MY, Heineman TC, Arvin AM. Varicella-zoster virus infection triggers formation of an interleukin-1beta (IL-1beta)-processing inflammasome complex. The Journal of Biological Chemistry. 2011;**286**(20):17921-17933

[59] Askari A, Nosratabadi R, Khaleghinia M, Zainodini N, Kennedy D, Shabani Z, et al. Evaluation of NLRC4, NLRP1, and NLRP3, as components of inflammasomes, in chronic hepatitis B virus-infected patients. Viral Immunology. 2016;**29**(9):496-501

[60] Yu X, Lan P, Hou X, Han Q, Lu N, Li T, et al. HBV inhibits LPS-induced NLRP3 inflammasome activation and IL-1beta production via suppressing the NF-kappaB pathway and ROS production. Journal of Hepatology. 2017;**66**(4):693-702

[61] Kolakofsky D, Kowalinski E, Cusack S. A structure-based model of RIG-I activation. RNA. 2012;**18**(12):2118-2127

[62] Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M, et al. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. Nature Immunology. 2004;**5**(7):730-737

[63] Fernandes-Alnemri T, Yu JW, Juliana C, Solorzano L, Kang S, Wu J, et al. The AIM2 inflammasome is critical for innate immunity to *Francisella tularensis*. Nature Immunology. 2010;**11**(5):385-393

[64] Pan X, Xu H, Zheng C, Li M, Zou X, Cao H, et al. Human hepatocytes express absent in melanoma 2 and respond to hepatitis B virus with interleukin-18 expression. Virus Genes. 2016;**52**(4):445-452

[65] Choubey D. Interferon-inducible Ifi200-family genes as modifiers of lupus susceptibility. Immunology Letters. 2012;**147**(1-2):10-17

[66] Uchida K, Akita Y, Matsuo K, Fujiwara S, Nakagawa A, Kazaoka Y, et al. Identification of specific autoantigens in Sjogren's syndrome by SEREX. Immunology. 2005;**116**(1):53-63

[67] Mondini M, Vidali M, Airo P, De Andrea M, Riboldi P, Meroni PL, et al. Role of the interferon-inducible gene IFI16 in the etiopathogenesis of systemic autoimmune disorders. Annals of the New York Academy of Sciences. 2007;**1110**:47-56

[68] Kerur N, Veettil MV, Sharma-Walia N, Bottero V, Sadagopan S, Otageri P, et al. IFI16 acts as a nuclear pathogen sensor to induce the inflammasome in response to Kaposi Sarcoma-associated herpesvirus infection. Cell Host & Microbe. 2011;**9**(5):363-375

[69] Dutta D, Dutta S, Veettil MV, Roy A, Ansari MA, Iqbal J, et al. BRCA1 regulates IFI16 mediated nuclear innate sensing of herpes viral DNA and subsequent induction of the innate inflammasome and interferon-beta responses. PLoS Pathogens. 2015;**11**(6):e1005030

[70] Wang J, Alexander J, Wiebe M, Jones C. Bovine herpesvirus 1 productive infection stimulates inflammasome formation and caspase 1 activity. Virus Research. 2014;**185**:72-76

[71] Chevaliez S, Pawlotsky JM. HCV genome and life cycle. In: Tan SL, editor. Hepatitis C Viruses: Genomes and Molecular Biology. Norfolk (UK): Horizon Bioscience; 2006

[72] MacCallum PR, Jack SC, Egan PA, McDermott BT, Elliott RM, Chan SW. Cap-dependent and hepatitis C virus internal ribosome entry site-mediated translation are modulated by phosphorylation of eIF2alpha under oxidative stress. The Journal of General Virology. 2006;**87**(Pt 11):3251-3262

[73] Jaafar ZA, Oguro A, Nakamura Y, Kieft JS. Translation initiation by the hepatitis C virus IRES requires eIF1A and ribosomal complex remodeling. eLife. 2016;**5**:e21198

[74] Bradrick SS, Walters RW, Gromeier M. The hepatitis C virus 3'-untranslated region or a poly(A) tract promote efficient translation subsequent to the initiation phase. Nucleic Acids Research. 2006;**34**(4):1293-1303

[75] Appel N, Schaller T, Penin F, Bartenschlager R. From structure to function: New insights into hepatitis C virus RNA replication. The Journal of Biological Chemistry. 2006;**281**(15):9833-9836

[76] Pradere JP, Kluwe J, De Minicis S, Jiao JJ, Gwak GY, Dapito DH, et al. Hepatic macrophages but not dendritic cells contribute to liver fibrosis by promoting the survival of activated hepatic stellate cells in mice. Hepatology. 2013;**58**(4):1461-1473

[77] Tanaka H, Fujita N, Sugimoto R, Urawa N, Horiike S, Kobayashi Y, et al. Hepatic oxidative DNA damage is associated with increased risk for hepatocellular carcinoma in chronic hepatitis C. British Journal of Cancer. 2008;**98**(3):580-586

[78] Di Bisceglie AM. Natural history of hepatitis C: Its impact on clinical management. Hepatology. 2000;**31**(4):1014-1018

[79] Nunez O, Fernandez-Martinez A, Majano PL, Apolinario A, Gomez-Gonzalo M, Benedicto I, et al. Increased intrahepatic cyclooxygenase 2, matrix metalloproteinase 2, and matrix metalloproteinase 9 expression is associated with progressive liver disease in chronic hepatitis C virus infection: Role of viral core and NS5A proteins. Gut. 2004; **53**(11):1665-1672

[80] Huang YS, Hwang SJ, Chan CY, Wu JC, Chao Y, Chang FY, et al. Serum levels of cytokines in hepatitis C-related liver disease: A longitudinal study. Zhonghua Yi Xue Za Zhi (Taipei). 1999;**62**(6):327-333

[81] Matsuzaki K. Modulation of TGF-beta signaling during progression of chronic liver diseases. Frontiers in Bioscience (Landmark Ed). 2009;(14):2923-2934

[82] Okumoto K, Hattori E, Tamura K, Kiso S, Watanabe H, Saito K, et al. Possible contribution of circulating transforming growth factor-beta 1 to immunity and prognosis in unresectable hepatocellular carcinoma. Liver International. 2004;**24**(1):21-28

[83] Teicher BA. Malignant cells, directors of the malignant process: Role of transforming growth factor-beta. Cancer Metastasis Reviews. 2001;**20**(1-2):133-143

[84] Gong G, Waris G, Tanveer R, Siddiqui A. Human hepatitis C virus NS5A protein alters intracellular calcium levels, induces oxidative stress, and activates STAT-3 and NF-kappa B. Proceedings of the National Academy of Sciences of the United States of America. 2001;**98**(17):9599-9604

[85] Qadri I, Iwahashi M, Capasso JM, Hopken MW, Flores S, Schaack J, et al. Induced oxidative stress and activated expression of manganese superoxide dismutase during hepatitis C virus replication: Role of JNK, p38 MAPK and AP-1. Biochemical Journal. 2004;**378**(Pt 3):919-928

[86] Moriya K, Fujie H, Shintani Y, Yotsuyanagi H, Tsutsumi T, Ishibashi K, et al. The core protein of hepatitis C virus induces hepatocellular carcinoma in transgenic mice. Nature Medicine. 1998;**4**(9):1065-1067

[87] Okuda M, Li K, Beard MR, Showalter LA, Scholle F, Lemon SM, et al. Mitochondrial injury, oxidative stress, and antioxidant gene expression are induced by hepatitis C virus core protein. Gastroenterology. 2002;**122**(2):366-375

[88] Gotoh M, Sakamoto M, Kanetaka K, Chuuma M, Hirohashi S. Overexpression of osteopontin in hepatocellular carcinoma. Pathology International. 2002;**52**(1):19-24

[89] Patouraux S, Bonnafous S, Voican CS, Anty R, Saint-Paul MC, Rosenthal-Allieri MA, et al. The osteopontin level in liver, adipose tissue and serum is correlated with fibrosis in patients with alcoholic liver disease. PLoS One. 2012;**7**(4):e35612

[90] Iqbal J, McRae S, Banaudha K, Mai T, Waris G. Mechanism of hepatitis C virus (HCV)-induced osteopontin and its role in epithelial to mesenchymal transition of hepatocytes. The Journal of Biological Chemistry. 2013;**288**(52):36994-37009

[91] Iqbal J, McRae S, Mai T, Banaudha K, Sarkar-Dutta M, Waris G. Role of hepatitis C virus induced osteopontin in epithelial to mesenchymal transition, migration and invasion of hepatocytes. PLoS One. 2014;**9**(1):e87464

[92] Basu A, Meyer K, Lai KK, Saito K, Di Bisceglie AM, Grosso LE, et al. Microarray analyses and molecular profiling of Stat3 signaling pathway induced by hepatitis C virus core protein in human hepatocytes. Virology. 2006;**349**(2):347-358

[93] Nishitsuji H, Funami K, Shimizu Y, Ujino S, Sugiyama K, Seya T, et al. Hepatitis C virus infection induces inflammatory cytokines and chemokines mediated by the cross talk between hepatocytes and stellate cells. Journal of Virology. 2013;**87**(14):8169-8178

[94] Hosomura N, Kono H, Tsuchiya M, Ishii K, Ogiku M, Matsuda M, et al. HCV-related proteins activate Kupffer cells isolated from human liver tissues. Digestive Diseases and Sciences. 2011;**56**(4):1057-1064

[95] Polyak SJ, Khabar KS, Rezeiq M, Gretch DR. Elevated levels of interleukin-8 in serum are associated with hepatitis C virus infection and resistance to interferon therapy. Journal of Virology. 2001;**75**(13):6209-6211

[96] Li K, Foy E, Ferreon JC, Nakamura M, Ferreon AC, Ikeda M, et al. Immune evasion by hepatitis C virus NS3/4A protease-mediated cleavage of the Toll-like receptor 3 adaptor protein TRIF. Proceedings of the National Academy of Sciences of the United States of America. 2005;**102**(8):2992-2997

[97] Spanakis NE, Garinis GA, Alexopoulos EC, Patrinos GP, Menounos PG, Sklavounou A, et al. Cytokine serum levels in patients with chronic HCV infection. Journal of Clinical Laboratory Analysis. 2002;**16**(1):40-46

[98] Ramadori G, Saile B. Inflammation, damage repair, immune cells, and liver fibrosis: Specific or nonspecific, this is the question. Gastroenterology. 2004;**127**(3):997-1000

[99] Antonelli A, Ferri C, Ferrari SM, Ghiri E, Goglia F, Pampana A, et al. Serum levels of proinflammatory cytokines interleukin-1beta, interleukin-6, and tumor necrosis factor alpha in mixed cryoglobulinemia. Arthritis and Rheumatism. 2009;**60**(12):3841-3847

[100] Basha HI, Subramanian V, Seetharam A, Nath DS, Ramachandran S, Anderson CD, et al. Characterization of HCV-specific CD4+Th17 immunity in recurrent hepatitis C-induced liver allograft fibrosis. American Journal of Transplantation. 2011;**11**(4):775-785

[101] Kasprzak A, Zabel M, Biczysko W, Wysocki J, Adamek A, Spachacz R, et al. Expression of cytokines (TNF-alpha, IL-1alpha, and IL-2) in chronic hepatitis C: Comparative hybridocytochemical and immunocytochemical study in children and adult patients. The Journal of Histochemistry and Cytochemistry. 2004;**52**(1):29-38

[102] Moschen AR, Fritz T, Clouston AD, Rebhan I, Bauhofer O, Barrie HD, et al. Interleukin-32: A new proinflammatory cytokine involved in hepatitis C virus-related liver inflammation and fibrosis. Hepatology. 2011;**53**(6):1819-1829

[103] Knolle PA, Gerken G. Local control of the immune response in the liver. Immunological Reviews. 2000;**174**:21-34

[104] Waris G, Siddiqui A. Hepatitis C virus stimulates the expression of cyclooxygenase-2 via oxidative stress: Role of prostaglandin E2 in RNA replication. Journal of Virology. 2005;**79**(15):9725-9734

[105] Choi J, Lee KJ, Zheng Y, Yamaga AK, Lai MM, Ou JH. Reactive oxygen species suppress hepatitis C virus RNA replication in human hepatoma cells. Hepatology. 2004; **39**(1):81-89

[106] Bowman T, Garcia R, Turkson J, Jove R. STATs in oncogenesis. Oncogene. 2000;**19**(21): 2474-2488

[107] Waris G, Turkson J, Hassanein T, Siddiqui A. Hepatitis C virus (HCV) constitutively activates STAT-3 via oxidative stress: Role of STAT-3 in HCV replication. Journal of Virology. 2005;**79**(3):1569-1580

[108] Poli G. Pathogenesis of liver fibrosis: Role of oxidative stress. Molecular Aspects of Medicine. 2000;**21**(3):49-98

[109] Vidali M, Tripodi MF, Ivaldi A, Zampino R, Occhino G, Restivo L, et al. Interplay between oxidative stress and hepatic steatosis in the progression of chronic hepatitis C. Journal of Hepatology. 2008;**48**(3):399-406

[110] Presser LD, McRae S, Waris G. Activation of TGF-beta1 promoter by hepatitis C virus-induced AP-1 and Sp1: Role of TGF-beta1 in hepatic stellate cell activation and invasion. PLoS One. 2013;**8**(2):e56367

[111] Szabo G, Chang S, Dolganiuc A. Altered innate immunity in chronic hepatitis C infection: Cause or effect? Hepatology. 2007;**46**(4):1279-1290

[112] Wang Y, Li J, Wang X, Ye L, Zhou Y, Thomas RM, et al. Hepatitis C virus impairs TLR3 signaling and inhibits IFN-lambda 1 expression in human hepatoma cell line. Innate Immunity. 2014;**20**(1):3-11

[113] Wang N, Liang Y, Devaraj S, Wang J, Lemon SM, Li K. Toll-like receptor 3 mediates establishment of an antiviral state against hepatitis C virus in hepatoma cells. Journal of Virology. 2009;**83**(19):9824-9834

[114] Loo YM, Owen DM, Li K, Erickson AK, Johnson CL, Fish PM, et al. Viral and therapeutic control of IFN-beta promoter stimulator 1 during hepatitis C virus infection. Proceedings of the National Academy of Sciences of the United States of America. 2006; **103**(15):6001-6006

[115] Saito T, Hirai R, Loo YM, Owen D, Johnson CL, Sinha SC, et al. Regulation of innate antiviral defenses through a shared repressor domain in RIG-I and LGP2. Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**(2):582-587

[116] Liu HM, Loo YM, Horner SM, Zornetzer GA, Katze MG, Gale Jr M. The mitochondrial targeting chaperone 14-3-3epsilon regulates a RIG-I translocon that mediates membrane association and innate antiviral immunity. Cell Host & Microbe. 2012;**11**(5):528-537

[117] Loo YM, Gale Jr M. Immune signaling by RIG-I-like receptors. Immunity. 2011;**34**(5): 680-692

[118] Gack MU, Shin YC, Joo CH, Urano T, Liang C, Sun L, et al. TRIM25 RING-finger E3 ubiquitin ligase is essential for RIG-I-mediated antiviral activity. Nature. 2007; **446**(7138):916-920

[119] Baril M, Racine ME, Penin F, Lamarre D. MAVS dimer is a crucial signaling component of innate immunity and the target of hepatitis C virus NS3/4A protease. Journal of Virology. 2009;**83**(3):1299-1311

[120] Horner SM, Liu HM, Park HS, Briley J, Gale Jr M. Mitochondrial-associated endoplasmic reticulum membranes (MAM) form innate immune synapses and are targeted by hepatitis C virus. Proceedings of the National Academy of Sciences of the United States of America. 2011;**108**(35):14590-14595

[121] Csak T, Ganz M, Pespisa J, Kodys K, Dolganiuc A, Szabo G. Fatty acid and endotoxin activate inflammasomes in mouse hepatocytes that release danger signals to stimulate immune cells. Hepatology. 2011;**54**(1):133-144

[122] Boaru SG, Borkham-Kamphorst E, Tihaa L, Haas U, Weiskirchen R. Expression analysis of inflammasomes in experimental models of inflammatory and fibrotic liver disease. Journal of Inflammation (Lond). 2012;**9**(1):49

[123] Shrivastava S, Mukherjee A, Ray R, Ray RB. Hepatitis C virus induces interleukin-1beta (IL-1beta)/IL-18 in circulatory and resident liver macrophages. Journal of Virology. 2013;**87**(22):12284-12290

[124] Sun Q, Gao W, Loughran P, Shapiro R, Fan J, Billiar TR, et al. Caspase 1 activation is protective against hepatocyte cell death by up-regulating beclin 1 protein and mitochondrial autophagy in the setting of redox stress. The Journal of Biological Chemistry. 2013;**288**(22):15947-15958

[125] Taxman DJ, Holley-Guthrie EA, Huang MT, Moore CB, Bergstralh DT, Allen IC, et al. The NLR adaptor ASC/PYCARD regulates DUSP10, mitogen-activated protein kinase (MAPK), and chemokine induction independent of the inflammasome. The Journal of Biological Chemistry. 2011;**286**(22):19605-19616

[126] Li YF, Nanayakkara G, Sun Y, Li X, Wang L, Cueto R, et al. Analyses of caspase-1-regulated transcriptomes in various tissues lead to identification of novel IL-1beta-, IL-18- and sirtuin-1-independent pathways. Journal of Hematology & Oncology. 2017;**10**(1):40

[127] Guarda G, Braun M, Staehli F, Tardivel A, Mattmann C, Forster I, et al. Type I interferon inhibits interleukin-1 production and inflammasome activation. Immunity. 2011; **34**(2):213-223

[128] Marcucci F, Mele A. Hepatitis viruses and non-Hodgkin lymphoma: Epidemiology, mechanisms of tumorigenesis, and therapeutic opportunities. Blood. 2011;**117**(6):1792-1798

[129] Ishizaka N, Ishizaka Y, Takahashi E, Tooda E, Hashimoto H, Nagai R, et al. Association between hepatitis C virus seropositivity, carotid-artery plaque, and intima-media thickening. Lancet. 2002;**359**(9301):133-135

[130] Adinolfi LE, Restivo L, Zampino R, Guerrera B, Lonardo A, Ruggiero L, et al. Chronic HCV infection is a risk of atherosclerosis. Role of HCV and HCV-related steatosis. Atherosclerosis. 2012;**221**(2):496-502

[131] Tillmann HL. Hepatitis C virus infection and the brain. Metabolic Brain Disease. 2004; **19**(3-4):351-356

[132] Chen KJ, Tseng CK, Chang FR, Yang JI, Yeh CC, Chen WC, et al. Aqueous extract of the edible Gracilaria tenuistipitata inhibits hepatitis C viral replication via cyclooxygenase-2 suppression and reduces virus-induced inflammation. PLoS One. 2013;**8**(2):e57704

[133] Wang Y, Gao J, Zhang D, Zhang J, Ma J, Jiang H. New insights into the antifibrotic effects of sorafenib on hepatic stellate cells and liver fibrosis. Journal of Hepatology. 2010;**53**(1):132-144

[134] Cohen-Naftaly M, Friedman SL. Current status of novel antifibrotic therapies in patients with chronic liver disease. Therapeutic Advances in Gastroenterology. 2011;**4**(6):391-417

[135] Sandler NG, Koh C, Roque A, Eccleston JL, Siegel RB, Demino M, et al. Host response to translocated microbial products predicts outcomes of patients with HBV or HCV infection. Gastroenterology. 2011;**141**(4):1220-1230. (e1-e3)

[136] Dammacco F, Tucci FA, Lauletta G, Gatti P, De Re V, Conteduca V, et al. Pegylated interferon-alpha, ribavirin, and rituximab combined therapy of hepatitis C virus-related mixed cryoglobulinemia: A long-term study. Blood. 2010;**116**(3):343-353

[137] Dinarello CA. A clinical perspective of IL-1beta as the gatekeeper of inflammation. European Journal of Immunology. 2011;**41**(5):1203-1217

[138] Uribe M, Go VL. Corticosteroid pharmacokinetics in liver disease. Clinical Pharmacokinetics. 1979;**4**(3):233-240

[139] Malekzadeh R, Nasseri-Moghaddam S, Kaviani MJ, Taheri H, Kamalian N, Sotoudeh M. Cyclosporin A is a promising alternative to corticosteroids in autoimmune hepatitis. Digestive Diseases and Sciences. 2001;**46**(6):1321-1327

[140] Johnson PJ, McFarlane IG, Williams R. Azathioprine for long-term maintenance of remission in autoimmune hepatitis. The New England Journal of Medicine. 1995; **333**(15):958-963

[141] Manns MP, Woynarowski M, Kreisel W, Lurie Y, Rust C, Zuckerman E, et al. Budesonide induces remission more effectively than prednisone in a controlled trial of patients with autoimmune hepatitis. Gastroenterology. 2010;**139**(4):1198-1206

[142] Larsen FS, Vainer B, Eefsen M, Bjerring PN, Adel Hansen B. Low-dose tacrolimus ameliorates liver inflammation and fibrosis in steroid refractory autoimmune hepatitis. World Journal of Gastroenterology. 2007;**13**(23):3232-3236

[143] Tacke F. Cenicriviroc for the treatment of non-alcoholic steatohepatitis and liver fibrosis. Expert Opinion on Investigational Drugs. 2018;**27**(3):301-311

[144] Rice LM, Padilla CM, McLaughlin SR, Mathes A, Ziemek J, Goummih S, et al. Fresolimumab treatment decreases biomarkers and improves clinical symptoms in systemic sclerosis patients. The Journal of Clinical Investigation. 2015;**125**(7):2795-2807

[145] Bansal R, Nagorniewicz B, Prakash J. Clinical advancements in the targeted therapies against liver fibrosis. Mediators of Inflammation. 2016;**2016**:7629724

[146] Kessoku T, Imajo K, Honda Y, Kato T, Ogawa Y, Tomeno W, et al. Resveratrol ameliorates fibrosis and inflammation in a mouse model of nonalcoholic steatohepatitis. Scientific Reports. 2016;**6**:22251

[147] Burmester GR, Panaccione R, Gordon KB, McIlraith MJ, Lacerda AP. Adalimumab: Long-term safety in 23 458 patients from global clinical trials in rheumatoid arthritis,

juvenile idiopathic arthritis, ankylosing spondylitis, psoriatic arthritis, psoriasis and Crohn's disease. Annals of the Rheumatic Diseases. 2013;**72**(4):517-524

[148] Tindall E. Celecoxib for the treatment of pain and inflammation: The preclinical and clinical results. The Journal of the American Osteopathic Association. 1999;**99** (11_suppl):S13-SS7

[149] Ko WK, Lee SH, Kim SJ, Jo MJ, Kumar H, Han IB, et al. Anti-inflammatory effects of ursodeoxycholic acid by lipopolysaccharide-stimulated inflammatory responses in RAW 264.7 macrophages. PLoS One. 2017;**12**(6):e0180673

[150] Liang TJ, Yuan JH, Tan YR, Ren WH, Han GQ, Zhang J, et al. Effect of ursodeoxycholic acid on TGF beta1/Smad signaling pathway in rat hepatic stellate cells. Chinese Medical Journal. 2009;**122**(10):1209-1213

[151] Hernandez E, Correa A, Bucio L, Souza V, Kershenobich D, Gutierrez-Ruiz MC. Pentoxifylline diminished acetaldehyde-induced collagen production in hepatic stellate cells by decreasing interleukin-6 expression. Pharmacological Research. 2002; **46**(5):435-443

[152] Austin AS, Mahida YR, Clarke D, Ryder SD, Freeman JG. A pilot study to investigate the use of oxpentifylline (pentoxifylline) and thalidomide in portal hypertension secondary to alcoholic cirrhosis. Alimentary Pharmacology & Therapeutics. 2004;**19**(1):79-88

[153] Raetsch C, Jia JD, Boigk G, Bauer M, Hahn EG, Riecken EO, et al. Pentoxifylline downregulates profibrogenic cytokines and procollagen I expression in rat secondary biliary fibrosis. Gut. 2002;**50**(2):241-247

Chapter 3

HCV Lymphotropism and Its Pathogenic Significance

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Additional information is available at the end of the chapter

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Abstract

Hepatitis C virus (HCV) is not only a hepatotropic but also a lymphotropic virus. Infection of the immune system appears to be a natural propensity of HCV and, as the accumulated data indicate, a common characteristic of both symptomatic and clinically silent but molecularly evident infection known as occult infection. The ability of HCV to infect cells of the immune system is consistent with a significantly greater prevalence of certain lymphoproliferative disorders in HCV-infected patients, such as mixed cryoglobulinemia and B-cell non-Hodgkin's lymphoma. This chapter recapitulates the approaches used to detect HCV and its replication within lymphoid cells, features of HCV compartmentalization in the lymphatic system and in different types of immune cells, and the cell culture models developed to study HCV lymphotropism. In addition, the characteristics of the molecules recently identified as those specifically mediating HCV entry leading to virus replication in B and T lymphocytes, which are distinct from those involved in virus entry to hepatocytes, are presented. Finally, the biological impact of HCV lymphotropism on the function of immune cells, virus persistence, and immune cell proliferation and lymphomagenesis is summarized.

Keywords: HCV immune cell tropism, T lymphocytes, B lymphocytes, monocytes, occult HCV infection, HCV persistence, immune cell functions, lymphomagenesis, cryoglobulinemia, B-cell non-Hodgkin's lymphoma

1. Introduction

The sequences of virological and immunological events leading to the pathological outcomes associated with hepatitis C virus (HCV) infection are not yet fully recognized. This is mainly because the onset of HCV infection rarely can be identified, the evolution to a symptomatic disease often takes decades, and the majority of predisposing factors and processes contributing to the development of the most important pathological consequences, such as cirrhosis,

hepatocellular carcinoma (HCC), and coinciding lymphoproliferative disorders, remain unknown. The fate of HCV during prodromal and convalescent phases of infection is also poorly recognized. Moreover, in addition to a symptomatic infection, which is normally accompanied by circulating HCV RNA and antibodies to HCV (anti-HCV), HCV can also persist as a clinically silent (occult) infection [1–3]. This infection is accompanied by very low levels of HCV RNA in serum (usually below 100–200 virus genome copies per mL), liver, and peripheral blood mononuclear cells (PBMCs), which are detectable with a significant difficulty, if at all, by clinical assays [4, 5]. This occult HCV infection (OCI) continues for decades after either spontaneous (self-limited) or antiviral therapy-induced resolution of hepatitis [1–4, 6–10]. OCI may have epidemiologic (e.g., contamination of blood and organ donations) and pathogenic (e.g., cryptogenic liver disease and oncogenicity) consequences which are not yet well recognized. Furthermore, experimental and clinical data indicate that HCV replicates not only in the liver but also in the lymphatic system, where it can modify development, proliferation, and function of immune cells [1, 2, 4, 5, 11–18]. It is also apparent that immune cells are reservoirs of persisting HCV where virus may hide from immune surveillance and elimination, similar to that in infections with other lymphotropic viruses [19–22]. The ability of HCV to infect cells of the immune system is consistent with a significantly greater prevalence of lymphoproliferative disorders in patients chronically infected with HCV, including mixed cryoglobulinemia (MC), B-cell non-Hodgkin's lymphoma (B-cell NHL), and marginal zone lymphoma [23–26]. Regression of these diseases in considerable numbers of patients treated with anti-HCV therapies is indicative of the direct role of HCV in the pathogenesis of those diseases [27, 28].

2. Identification of lymphotropic HCV replication

The current approach to the diagnosis of HCV infection, which typically includes testing of only serum samples but not samples from liver or otherwise easily accessible PBMC, using assays detecting only HCV RNA-positive (also termed as non-replicative, genomic, or vegetative) strand and anti-HCV, is an obstacle in the precise determination of HCV clearance, that is, cure. These limitations are also a source of controversies regarding the natural history and the longevity of infection, as well as the sites of virus persistence [29–33]. Consequently, patients who are considered free of infection may produce low levels of biologically competent virus for an extended time after vanishing of symptoms and biochemical normalization of liver function achieved due to either spontaneous resolution or clinically apparent sustained virologic response (SVR) to antiviral treatment [1, 4–6, 8, 9]. Testing of serial samples of serum or plasma and, in particular, PBMC collected a few months apart increases the detection of low levels of HCV RNA during follow-up, even when clinical assays of moderate or low sensitivity are applied. This is due to the fluctuating level of circulating virus during OCI that can temporarily increase to the levels detectable by these assays [2, 4, 5].

Identification of HCV infection in immune cells is not just about the mere detection of virus RNA-positive strand, since the occurrence of this strand alone may reflect incidental cell surface attachment or cellular uptake of virus or its genomic material. A combination of a few

approaches has been applied to credibly detect replicating HCV in immune cells [4]. They involve the documentation of (1) HCV RNA-negative (also termed as replicative or anti-genomic) strand [1, 6, 9, 34]; (2) viral structural and/or, preferentially, nonstructural proteins within the cytoplasm of infected cells [15, 34]; (3) distinctive HCV variants in total PBMC or their cell subsets when compared to those in plasma or in liver of infected patients, or emergence of HCV variants in cultured cells which are distinct from those occurring in inocula used to infect them [15, 34]; (4) susceptibility of infected cells to *ex vivo* treatment with interferon alpha (IFN-α) [6, 35] or direct acting antivirals (DAAs) [9, 34]; (5) transmission of infection to virus-naïve cells by cell-free supernatants from primary-cultured cells or *de novo*-infected cultures [34, 35]; (6) display of biophysical properties of virions by viral particles released during culture of infected cells [35], and (7) visualization of HCV virions released from infected cells by immunogold electron microscopy (IEM) with virus envelope-specific antibody [6, 35]. However, although the immune cells can support the complete cycle of HCV replication and production of biologically competent virion particles, the virus load per cell and the level of virus replication in either *in vivo*- or *in vitro*-infected immune cells are much lower than those seen in the HCV JFH-1 (Japanese fulminant hepatitis-1)-Huh7.5 cell infection system. Also, for comparison, the loads of HCV RNA per cell in total PBMC were found to be lower than those per hepatocyte in chronic hepatitis C (CHC), but comparable to each other during OCI [7]. Overall, investigations of HCV lymphotropism remain challenging and require highly sensitive detection techniques and meticulous approaches.

3. HCV compartmentalization in the immune system

HCV displays a remarkable genetic variability and typically exists in an infected host as a heterologous population of closely related subpopulations of viral particles carrying slightly different genomic sequences, called collectively as quasispecies. The 5′-untranslated region (5′-UTR) of virus genome contains an internal ribosome entry site (IRES) essential for viral RNA translation. This sequence is highly conserved among different HCV genomes, and arise of variants within this region is usually an indicator of a sustained virus change. HCV derived from extrahepatic locations tends to display variations in the IRES sequence when compared to the genomes from plasma and liver [5, 36]. Some of these substitutions are located at particular nucleotide positions, suggesting that they may reflect virus adaptation to replication in a non-hepatic milieu. In addition, variants within the hypervariable region-1 (HVR1) of the virus E2 protein, although much more common than those in the 5′-UTR, may also reflect the site-restricted replication of HCV variants. Overall, compartmentalization of HCV variants in immune cells is considered to be a reliable indicator of hepatocyte-independent virus replication [36].

The existence of HCV quasispecies with affinity to immune cells has been suggested shortly after HCV discovery [37–39]. Evidence for lymphotropic HCV variants was found in patients with CHC, acute hepatitis C, as well as in asymptomatic individuals with persistent OCI [4, 14, 15, 40–47]. Analyses of HCV variants residing in PBMC by clonal sequencing and single-stranded conformational polymorphism (SSCP) revealed features which argued against the possibility of carry-over of variants from plasma-derived HCV RNA or from

virus nonspecifically attached to the cell surface [5–9, 13, 15, 42, 48, 49]. Furthermore, HCV variants from lymphoid cells were genetically related, but distinct from those occurring in serum or liver. In some instances, HCV quasispecies identified in lymphoid cells were detectable in serum but not in liver tissue, indicating that the majority of circulating virus could be of extrahepatic origin. In this regard, the analysis of the HVR1 from liver, PBMC, and serum showed that certain virus variants occurring in serum resided only in PBMC but not in the liver [42, 44]. Moreover, HCV quasispecies from one cell type, for example, $CD8^+$ T lymphocytes, were statistically more genetically like one another when compared to variants from other immune cell subsets, such as $CD4^+$ T cells. In one pertinent study, HCV RNA sequences carried by $CD8^+$ T lymphocytes were phylogenetically clustered close to one another, but not to those detected in $CD4^+$ T cells or $CD19^+$ B cells [48]. It has been found that certain sequence polymorphisms within the IRES of the 5′-UTR of the HCV genomes originating from lymphoid cells coincided with a different IRES translational activity which could promote HCV replication in cells carrying those variations [36, 50]. Similarly, it was demonstrated using liver-derived hepatoma Huh7 cells that HCV IRES variants originating from plasma displayed a significantly higher translational activity than those from HCV residing in B cells [51]. On the other hand, the IRES variants of virus replicating in B cells displayed a similarly low translational efficiency in Raji and Daudi B-cell lines as well as in hepatoma Huh7 cells, suggesting not only their extrahepatic origin but also a low capacity to replicate in B cells [51].

As already alluded to, the studies on HCV compartmentalization in the immune system demonstrated the existence of replicating virus in all the main subsets of circulating lymphomononuclear cells, including B cells, T lymphocytes, and monocytes [1, 13, 15, 42, 52]. There is also evidence for HCV replication in other immune cell types, such as dendritic cells (DCs) [53, 54]. In one of our studies, the virus load and the level of HCV replication were quantified in total PBMC as well as in affinity-purified cell subsets from these PBMCs, including $CD4^+$ and $CD8^+$ T lymphocytes, B cells, and monocytes from patients with CHC or OCI [15]. This investigation showed significant differences in the level of immune cell subset infection between patients with CHC and OCI with overall greater HCV loads in immune cells in CHC compared to those with OCI. In addition, monocytes carried the greatest HCV amounts in CHC, while B cells tended to contain the highest virus loads, and monocytes were the least frequently infected in OCI. Interestingly, while the total PBMCs were HCV RNA nonreactive in some individuals, the immune cell subsets isolated from these PBMCs clearly displayed virus RNA and its replicative strand, suggesting preferential or exclusive infection of the particular immune cell subset. This also indicated that the testing of total PBMC may not always identify residing virus and, thus, the analysis of individual immune cell types should be considered. In this study, HCV replication in immune cells was ascertained by the detection of (1) HCV RNA replicative (negative) strand, (2) HCV nonstructural 5a protein (NS5A), and (3) HCV variants distinct from those found in plasma of the same patients. In addition, immune cells were exposed *ex vivo* to a T-cell-stimulating mitogen in the presence of human recombinant interleukin-2 (IL-2) to augment HCV replication. We previously showed that such a treatment increases HCV replication in immune cells and improves detection of virus [15, 55]. Overall,

the study identified the main immune cell types involved in HCV infection in CHC and OCI and demonstrated that the immune system supports HCV replication regardless of the clinical appearance of infection.

It should be mentioned that the identification of HCV in the lymphatic system is not limited only to PBMC. HCV genomes and its proteins were also demonstrated in lymph nodes and bone marrow [47, 56]. In regard to lymph nodes, replicating HCV genomes and virus core and NS3 proteins were detected within biopsied B-cell-rich lymphoid follicles from patients with CHC [47]. In one study, not only did B cells appear to be the primary site of HCV infection in this secondary lymphoid tissue, but clonal sequencing analyses also indicated that in certain patients, HCV residing in lymph node-derived B cells could contribute up to 40% of the total level of viremia [47]. Furthermore, it is of note that HCV RNA sequences found in cerebrospinal fluid of patients co-infected with human immunodeficiency virus type 1(HIV-1) were identified to be more similar to virus sequences in PBMC and lymph nodes than to those in plasma. This raised a possibility that cells of the monocyte/macrophage lineage may carry HCV into the brain, and that resident microglial cells maintain its replication independently of the liver [56, 57]. In addition, HCV RNA-positive and -negative strands, as well as HCV structural and nonstructural proteins, were readily detectable in $CD34^+$ hematopoietic progenitor cells in the bone marrow of patients with CHC [58], reinforcing the notion of extrahepatic HCV replication. However, there was no evidence of primary $CD34^+$ cells from healthy individuals supporting *de novo* HCV infection. The same study showed that $CD34^+$ cells from CHC patients could release HCV RNA into culture supernatant, linking the development of $CD34^+$ cells to their susceptibility to HCV.

4. Replication of HCV in primary immune cells and cultured immune cell lines

Direct support for the inherent propensity of HCV to enter and propagate in cells of the immune system stems from *in vitro* studies where HCV-susceptible stable human lymphocytic cell lines, normal human PBMC, or primary immune cell subsets isolated from PBMC were exposed to HCV. In this regard, it is important to note that only authentic, patient-derived virus, but not laboratory-constructed recombinant HCV clones, including HCV JFH-1, infect either primary immune cells or susceptible immune cell lines [59, 60]. Hence, it was reported that HCV carried in the serum or plasma of HCV-positive patients was infectious to Raji and Daudi B-cell lines [18, 61] and to T-cell lines, such as Molt-4 [62–64], HPB [65] and Jurkat (all derived from patients with acute T-lymphoblastic leukemia) [63, 64], and pre-stimulated PM1 (originated from acute cutaneous T-cell lymphoma) [63, 64]. It was also shown that HCV released from SB, a B-cell line established from the splenocytes of an HCV-positive patient with type II MC and monocytoid lymphoma [66], was infectious to human primary $CD4^+$ T cells [67], and Molt-4 and Jurkat T cells [68]. Others have demonstrated the ability of primary lymphoid cells from healthy individuals, including total PBMC [11, 43, 52], T lymphocytes [6, 15, 35], B cells [8, 15, 51, 69, 70], and monocytes/macrophages [15, 71] to support HCV infection.

The studies from our laboratory showed that authentic HCV of different genotypes can infect total T cells enriched in culture from PBMC of healthy donors by their intermittent stimulation with phytohemagglutinin (PHA) in the presence of human recombinant IL-2 [6, 34, 35, 63, 64]. Replication and secretion of infectious HCV virions in this system were ascertained by the detection of (1) HCV RNA replicative strand and NS5a and/or core protein in infected cells [6, 34, 35, 63], (2) the emergence of HCV variants not existing in inocula used to infect the cells [6, 34], (3) the release of HCV RNA-reactive particles with buoyant density and ultrastructural properties of virions [34, 35], (4) virions released by infected cells via IEM [6, 34], and (5) the serial passage of HCV produced by the *de novo*-infected cells in virus-naïve primary T cells [35]. Moreover, the *de novo* HCV infection was inhibited by IFN-α- [35] and HCV-specific protease inhibitor Telaprevir [34, 63]. Furthermore, the system was adapted to include readily available Molt-4 and Jurkat T cells as infection targets, making it independent from freshly isolated human PBMC [63, 64]. In this model, the infection of essentially intact cells with unmodified, naturally occurring HCV allowed for the determination of (1) infectivity of low levels of HCV persisting in the course of OCI [6], (2) CD5 as the T-cell-specific receptor that mediates infection of the cells with patient-derived HCV [63], (3) differential expression of candidate HCV receptors in human T cells prone or resistant to infection with authentic HCV [64], and (4) an impact of infection with HCV on the suppression of $CD4^+$ T lymphocyte proliferation [72]. In the most recent study [34], the same infection system was also applied to recognize quantitative differences between $CD4^+$ and $CD8^+$ T-cell subsets in the level of HCV replication and to define properties of the virus produced by these cells. These investigations showed that although HCV replicates in both cell subtypes, the level of HCV replication in $CD4^+$ T cells was significantly higher than that in $CD8^+$ T cells. Intracellular HCV NS5a and core proteins were displayed at a similar frequency in both subtypes, that is, in 0.9 and 1.2% of $CD4^+$ and $CD8^+$ cells, respectively. Double staining for HCV NS5a protein and CD4 or CD8 T-cell differentiation markers provided conclusive evidence that virus replicated in both cell types. In addition, virus produced by $CD4^+$ and $CD8^+$ cells displayed different biophysical properties than those characterizing viral particles occurring in plasma used to infect the cells, confirming that new virus was produced, and the same virus was infectious to naïve $CD4^+$ of $CD8^+$ T cells isolated from a healthy donor. Remarkably, the data obtained from the *in vitro* infection of $CD4^+$ and $CD8^+$ T cells were comparable to those characterizing the infection of primary $CD4^+$ and $CD8^+$ T cells isolated from HCV-infected patients [15], although the level of HCV replication tended to be higher in *in vitro* than in *in vivo* conditions. Since HCV-specific T-cell effector activity is considered to be a principal factor underlying the pathogenesis of CHC as well as the resolution of hepatitis [73, 74], HCV infection of the T cells may have a direct impact on the virus-specific immune T-cell-depended responses and, in consequence, advance both virus infection and disease process.

5. Molecules mediating entry leading to HCV replication in immune cells

The HCV envelope is composed by two glycoproteins, E1 and E2. These proteins are primarily responsible for the virion attachment to the cell surface molecules serving as receptors and for the subsequent steps of viral entry [75]. The ability of HCV to infect human cells has

been interpreted almost exclusively in the context of the interactions between HCV JFH-1 strain, related strains, or pseudoparticles and hepatocyte-like Huh7 cells or their subclone Huh7.5. Based on these studies, tetraspanin CD81 [76], glycosaminoglycans [77], low-density lipoprotein receptor (LDL-R) [77], scavenger receptor class B-type 1 (SR-B1) [76, 78], the tight junction protein claudin-1 (CLDN-1) [79], occludin (OCLN) [80–82], and co-factors, such as epidermal growth factor receptor (EGFR) and ephrin receptor A2 (EphA2) [83], have been proposed to contribute either directly or indirectly to HCV entry into hepatocytes. In addition, the Niemann-Pick C1-like 1 (NPC1L1) cholesterol absorption receptor and transferrin receptor 1 (TfR1) have been implicated in HCV entry [84, 85]. However, the degree to which these individual molecules participate in HCV infection of normal human hepatocytes by naturally occurring virus requires validation, particularly since the majority of these molecules are ubiquitously displayed on many cell types. On the other hand, molecules determining HCV lymphotropism remained entirely unknown until recently.

An important finding toward the recognition of virological determinants mediating HCV lymphotropism was recently provided which showed that a distinct virus subpopulation capable of encoding particular E1E2 (envelope) epitopes might be responsible for the infection of B lymphocytes [86]. Isolated HCV E1E2 glycoproteins from patient B cells were able to confer the ability to enter and replicate in B cells to a non-lymphotropic HCV JFH-1 strain, as demonstrated by the detection of viral RNA and proteins within those cells. Interestingly, the B-cell tropism coincided with a loss of the JFH-1 strain ability to infect liver cancer-derived, hepatocyte-like Huh7 cells, implying that a lymphotropic variant constituted a separate population of viral particles displaying unique E1E2 envelope specificity. These results also supported a notion that a receptor for HCV on B cells is distinct from that on hepatocytes.

The recent finding that a co-stimulatory receptor B7.2 (CD86) is involved in the infection of human memory B cells by the abovementioned HCV SB strain [66] substantiated the involvement of a hepatocyte-distinct receptor in HCV lymphotropism [87] (**Table 1**). The study showed that the virus E1E2 envelope and 5′-UTR sequences determine lymphotropism of the SB strain and that silencing of the virus sensor retinoic acid-inducible gene I (RIGI) or overexpression of micrcoRNA-122 permitted the persistence of viral replication in B cells. Furthermore, the interaction of the SB virus E2 protein with the cell B7.2 protein reduced the surface display of B7.2 on memory B cells and inhibited their function. Interestingly, it was also found that memory B cells in HCV-infected patients expressed significantly lower levels of surface B7.2 when compared to those in HCV-negative individuals, but they carried significantly higher levels of HCV RNA than naïve B cells derived from HCV-positive patients. This comprehensive study provided important data on several aspects of HCV B-cell tropism and its potential functional and pathological consequences.

By applying the HCV-human T-cell infection system established in our laboratory [35], it was uncovered that CD5, a lymphocyte-specific 67-kDa glycoprotein belonging to the scavenger receptor cysteine-rich family, is essential for the infection of human T cells by authentic, patient-derived HCV [63] (**Table 1**). This work also demonstrated that CD81 likely contributes as a co-receptor, since both anti-CD5 and anti-CD81 monoclonal antibodies inhibited HCV infection. However, only CD5-positive T cells were susceptible to infection [63]. Thus, it appears that while CD81 contributes to the broad recognition of cells by HCV, CD5 facilitates HCV tropism toward T lymphocytes. In this context, primary human hepatocytes and hepatoma cell lines

HCV	HCV genotype	Immune cell target	Receptor molecule	Receptor properties	Receptor physiological function	Expected consequences of HCV-receptor interaction
Authentic (wild-type), patient plasma-derived (Ref. [63])	1–4	T cell	CD5	67-kDa glycoprotein, cysteine-rich scavenger receptor superfamily	Co-stimulatory molecule modulating positively or negatively intracellular signaling pathways induced by the antigen-specific T and B cell receptors	Unknown
SB variant, B-cell lymphoma-derived (Ref. [87])	2b	B cell	B7.2 (CD86)	60–100-kDa glycoprotein, immunoglobulin superfamily	Co-stimulatory molecule interacting with CD28 for T-cell activation and CTLA4 for T-cell immune regulation	Reduction of B7.2 on memory B cells and inhibition of the cells function in HCV-positive patients

Table 1. Receptor molecules mediating HCV entry and replication in human lymphocytes.

were found to express trace amounts of CD5 mRNA but not protein [63, 64], clearly indicating that HCV utilizes different receptors to enter different cell targets. This was confirmed in a subsequent study that investigated the expression of hepatocyte HCV candidate receptors on human T lymphocytes prone or resistant to HCV infection with authentic virus [64]. The expression of SR-B1, occludin, CLDN-1 and -6, CD5, and CD81 was determined by real-time polymerase chain reaction (RT-PCR), and their proteins quantified by immunoblotting in T-cell lines found to be prone or resistant to HCV infection, PBMC, primary T cells and $CD4^+$ and $CD8^+$ T-cell subsets, and compared to hepatoma-derived, well-differentiated Huh7.5 and HepG2 cells. SR-B1 protein was found in T and hepatoma cell lines but not on PBMC or primary T lymphocytes, while CLDN-1 was detected only in HCV-resistant (when unstimulated) PM1 cell line and hepatoma cell lines, and CLDN-6 was equally expressed across all cells investigated. OCLN protein occurred in HCV-susceptible Molt-4 and Jurkat T cells and in trace amounts in primary T cells, but not in PBMC. CD5 was expressed by HCV-prone T-cell lines, primary T cells, and PBMC, but not by non-susceptible T and hepatoma cell lines, while CD81 was detected in all cell types except HepG2. Furthermore, knocking down OCLN in a virus-prone T-cell line inhibited HCV infection, while *de novo* infection down-regulated both OCLN and CD81 and upregulated CD5 without modifying SR-B1 expression. Overall, while no association between SR-B1, CLDN-1, or CLDN-6 and the susceptibility to HCV was found, CD5 and CD81 expression coincided with virus lymphotropism and that of OCLN with permissiveness of T-cell lines, but seemingly not primary T cells. This study narrowed the range of candidate entry receptors utilized by HCV to infect T lymphocytes

among those already uncovered using laboratory-grown HCV and Huh7.5 cells and confirmed that authentic HCV utilizes different receptors to enter hepatocytes and lymphocytes. The use of different receptors to infect multiple cell types is not uncommon among viruses. For example, HIV-1 uses CD4 to infect T cells but predominantly CCR5 to infect macrophages [88, 89]. The measles virus utilizes SLAM to infect lymphocytes, macrophages, and DC, but also infects SLAM-negative epithelial and endothelial cells [90, 91]. Also, EBV uses CD21 to infect B cells and DC, but this virus also replicates in epithelial cells which do not transcribe CD21 [92, 93]. In summary, CD5 was the first identified cell surface receptor that governs human cell permissiveness to HCV in a cell-type-specific manner. Since CD5 is also expressed on a minor subset of human pro-B lymphocytes [94], this molecule may potentially contribute to HCV entry to these B cells. Taken together, the results from several studies imply that cell-type-specific surface molecules, rather than a combination of molecules naturally displayed on many diverse cell types, mediate HCV tropism toward lymphocytes.

6. Functional and pathological consequences of HCV lymphotropism

The replication of HCV in immune cells, even at low levels, has a potential to affect their function, proliferation kinetics, and yield pathological outcomes, similar to infections with other lymphotropic viruses. Although the data remain overall sparse, there is a meaningful progress in some aspects. In particular, the study of a lymphotropic HCV SB strain brought the recognition of various specific mechanisms by which HCV may modify immune cell proliferation and function [95]. Among others, it has been shown that the transient infection of primary $CD4^+$ T cells and selected T-cell lines with the SB strain distorted the IFN-γ/STAT-1/T-bet signaling leading to the inhibition of IFN-γ production [67, 96]. It was also reported that infection with this strain suppressed the proliferation of primary $CD4^+$ T cells and their differentiation toward the Th1 lineage, as well as inhibited Molt-4 T-cell proliferation while enhancing their CD95 (Fas)-mediated apoptosis [68]. A study from our group showed that naturally occurring, patient-derived HCV inhibited the proliferation of primary $CD4^+$, but not $CD8^+$ T cells, without augmenting cell death [72]. Interestingly, the results also suggested that just an exposure to authentic HCV in the absence of molecularly evident viral replication might be sufficient to inhibit $CD4^+$ T-cell proliferation. It has also been shown that HCV core protein is capable of the transcriptional activation of the IL-2 promoter in T cells [97] and could modulate T-cell responses by inducing spontaneous and T-cell receptor (TCR)-mediated oscillations of calcium ions [98]. Others demonstrated that HCV core protein upregulated the expression of anergy-related genes in Jurkat T cells stably expressing this protein, and this coincided with the activation of nuclear factor of activated T cells (NFAT) and suppression of the IL-2 promoter [99]. In addition, it was reported that the direct binding of HCV core protein to complement receptor, gC1qR, on $CD4^+$ and $CD8^+$ T cells upregulated the expression of programmed death-1 (PD-1). This was accompanied by the dysregulation of T-cell activation, proliferation, and apoptosis [100]. These alterations were restored by blocking the engagement of the PD-1 and programmed death ligand-1 (PDL-1) pathway.

Interesting findings have been recently reported regarding the effect of exposure of primary human T cells to authentic, plasma-occurring HCV and to virus E2 protein or E2 encoding RNA on the TCR-signaling pathways [101]. It is of note that TCR signaling is critical for the normal functioning of $CD4^+$ and $CD8^+$ T cells, including their differentiation, activation, proliferation, and effector functions. The study showed that HCV interferes with TCR signaling and impairs T-cell activation *via* two distinct mechanisms. The first included intracellular processing of virus E2 coding RNA into a shorter, 51 nucleotide-long RNA fragment, predicted to be a dicer substrate. This virus-derived sequence targets a regulatory phosphatase involved in Src kinase signaling (abbreviated as PTPRE), subsequently inhibiting TCR signaling. In the second mechanism, the lymphocyte-specific Src kinase (Lck) phosphorylated HCV E2 protein, resulting in the inhibition of nuclear transportation of activated NFAT and in turn reducing TCR activation. It was concluded that HCV particles deliver viral RNA and E2 protein to T cells and that the highly conserved motifs of both RNA and protein inhibit TCR signaling, contributing to T-cell dysfunction and virus persistence. A second study from the same group showed that PTPRE levels are significantly reduced in liver tissue and PBMC of HCV-infected patients compared to those of uninfected controls [102]. It was demonstrated that a deficiency in PTPRE expression impaired antigen-specific TCR signaling, while antiviral therapy rescued the enzyme expression in PBMC and restored antigen-specific TCR signaling. Overall, the data indicated that HCV infection of T cells hinders TCR signaling and that short, regulatory HCV RNA sequences intracellularly derived from HCV genomic RNA are crucial in this process. The data from our studies showing that the authentic, patient-derived HCV can productively infect $CD4^+$ and $CD8^+$ T cells both *in vivo* [15] and *in vitro* [34] provide an indispensable link between HCV infection and the findings reported in the above studies.

The propensity of HCV to infect B cells is consistent with a significantly greater prevalence of certain B-cell proliferative disorders, particularly B-cell NHL, in HCV-infected individuals. Several lines of evidence support a link between HCV infection and B-cell NHL. These include (1) a strong epidemiological association of B-cell NHL with persistent HCV infection [25], (2) clinical data demonstrating that successful anti-HCV therapy often results in the remission of B-cell NHL [27, 28], (3) experimental data showing that transgenic mice expressing the full-length HCV genome specifically in B cells spontaneously develop B-cell NHL [103], and (4) both *in vivo* and *in vitro* data indicating that B lymphocytes are susceptible to infection and capable of supporting HCV replication [15, 18].

The mechanisms of lymphomagenesis associated with HCV infection were investigated by several groups, and a few concepts have been proposed (reviewed in [17]). However, it should be taken into consideration the fact that MC frequently precedes the development of B-cell NHL in HCV-infected individuals, indicating that MC might be a transitional step in the progression to lymphoma [104]. The proposed mechanisms of the pathogenesis of HCV-associated of lymphoma (i.e., lymphomagenesis) can be divided into two main categories. One includes the protracted stimulation of B cells by HCV antigens leading to pathologically augmented proliferation of the cells; a process that may involve different intracellular mechanisms. Another category relates to direct HCV infection and replication within B cells causing alterations in B-cell receptor (BCR) signaling or mutagenic changes in the cellular DNA

leading to oncogenic transformation of the infected B cells. It should be noted that BCR signaling is essential for the development and activation of normal B cells and is recognized as a critical pathway in the pathogenesis of several B-cell malignancies [105]. The expected role of HCV antigens in the stimulation of B cells is exemplified by the findings from the *in vitro* studies demonstrating that the engagement of CD81 on B cells by HCV E2 protein, which binds with a high affinity to the large extracellular loop of CD81, resulted in cell activation without BCR involvement [106]. This study also reported that the majority of HCV-infected patients display circulating B cells with an activated phenotype that disappears following treatment with HCV antivirals. Another relevant study showed that the exposure of $CD14^+$ cells to recombinant HCV core protein augmented the production of IL-6 through Toll-like receptor-2 pathway which coincided with an increased B-cell proliferation [107]. With regard to the second category of the mechanisms, a recent study confirmed the expression of HCV proteins in B cells from infected patients as well as in isolated B cells exposed *in vitro* to authentic patient-derived HCV and established that these cells have upregulated BCR signaling [18]. The study demonstrated a hierarchy of molecular events in which the overexpression of HCV nonstructural protein NS3/4a interferes with checkpoint kinase 2 (CHK2), which in turn phosphorylates Hu-antigen R (HuR) that regulates its target mRNAs that encode proteins of stress response, cell proliferation, and tumorigenesis [108]. The BCR-signaling pathway was found to be the top-ranked pathway showing an increased association with activated HuR and being upregulated by NS3/4a overexpression. This study revealed an important biological role of NS3/4a in the regulation of BCR signaling and advanced understanding of the molecular processes underlying HCV-associated B-cell proliferation. In a study from another group, it has also demonstrated that HCV NS3/4a protein also interacts with ATM (ataxia telangiectasia mutated) and inhibits DNA repair in non-lymphoid cells [109]. It is known that the prior mentioned CHK2 is the key downstream molecule of ATM and is a key sensor of DNA damage. Therefore, it is possible that the augmented BCR signaling and enhanced mutagenesis may cooperate in the induction of pro-oncogenic changes in HCV-infected B cells. In addition to the studies acknowledged earlier, the mechanisms of HCV infection-associated lymphoproliferation and lymphomagenesis have been, and continue to be, the subjects of several other studies (reviewed in [17, 110, 111]).

7. Conclusions

The authenticity of HCV lymphotropism is now evident, and sizable progress was made in deciphering this event. The accumulated experimental, clinical, and, to some degree, epidemiological data indicate that HCV not only propagates in the liver but also within the immune system, where the virus can modify the proliferation and function of affected cells and induce lymphoproliferative disease. It also became apparent that immune cells constitute a site of long-term persistence of HCV, where virus hides from immune surveillance and elimination, similar to other lymphotropic viruses. Accumulated evidence indicates that HCV replication in immune cells is a constant feature of both symptomatic and occult infections, although the degree to which individual immune cell types support infection significantly varies between patients. Despite recent progress, there remains a substantial void in the data

on several fundamental aspects of HCV lymphotropism and the biological effects of lymphotropic variants. In addition, the range of immunological and pathological implications of HCV lymphotropism, in particular, the contribution to aiding virus persistence and protraction of liver disease, and the mechanisms of virus-triggered lymphoproliferation, required further studies. The progress achieved to date provides strong justification for the need to intensify research in this field.

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Conflict of interest

Nothing to declare.

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References

[1] Pham TNQ, MacParland SA, Mulrooney PM, Cooksley H, Naoumov NV, Michalak TI. Hepatitis C virus persistence after spontaneous or treatment-induced resolution of hepatitis C. Journal of Virology. 2004;**78**:5867-5874

[2] Radkowski M, Gallegos-Orozco JF, Jablonska J, Colby TV, Walewska-Zielecka B, Kubicka J, Wilkinson J, Adair D, Rakela J, Laskus T. Persistence of hepatitis C virus in patients successfully treated for chronic hepatitis C. Hepatology. 2005;**41**:106-114

[3] Castillo I, Rodriguez-Inigo E, Lopez-Alcorocho JM, Pardo M, Bartolome J, Carreño V. Hepatitis C virus replicates in the liver of patients who have a sustained response to antiviral treatment. Clinical Infectious Diseases. 2006;**43**:1277-1283

[4] Pham TNQ, Coffin CS, Michalak TI. Occult hepatitis C virus infection: What does it mean? Liver International. 2010;**30**:502-511

[5] Di Liberto G, Roque-Afonso AM, Kara R, Ducoulombier D, Fallot G, Samuel D, Feray C. Clinical and therapeutic implications of hepatitis C virus compartmentalization. Gastroenterology. 2006;**131**:76-84

[6] MacParland SA, Pham TNQ, Guy CS, Michalak TI. Hepatitis C virus persisting after clinically apparent sustained virological response to antiviral therapy retains infectivity in vitro. Hepatology. 2009;**49**:1431-1441

[7] Pham TNQ, Coffin CS, Churchill ND, Urbanski SJ, Lee SS, Michalak TI. Hepatitis C virus persistence after sustained virological response to antiviral therapy in patients with or without past exposure to hepatitis B virus. Journal of Viral Hepatitis. 2012;**19**:103-111

[8] Chen AY, Zeremski M, Chauhan R, Jacobson IM, Talal AH, Michalak TI. Persistence of hepatitis C virus during and after otherwise clinically successful treatment of chronic hepatitis C with standard pegylated interferon α-2 and ribavirin therapy. PLoS One. 2013;**8**:e80078

[9] Chen AY, Hoare M, Shankar AN, Allison M, Alexander GJ, Michalak TI. Persistence of hepatitis C virus traces after spontaneous resolution of hepatitis C. PLoS One. 2015;**10**:e0140312

[10] Elmasry S, Wadhwa S, Bang BR, Cook L, Chopra S, Kanel G, Kim B, Harper T, Feng Z, Jerome KR, Kahn JA, Saito T. Detection of occult hepatitis C virus infection in patients who achieved a sustained virologic response to direct-acting antiviral agents for recurrent infection after liver transplantation. Gastroenterology. 2017;**152**:550-553

[11] Zignego AL, Macchia D, Monti M, Thiers V, Mazzetti M, Foschi M, Maggi E, Romagnani S, Gentilini P, Bréchot C. Infection of peripheral mononuclear blood cells by hepatitis C virus. Hepatology. 1992;**15**:382-386

[12] Laskus T, Radkowski M, Wang LF, Vargas H, Rakela J. The presence of active hepatitis C virus replication in lymphoid tissue in patients coinfected with human immunodeficiency virus type 1. Journal of Infectious Diseases. 1998;**178**:1189-1192

[13] Ducoulombier D, Roque-Afonso AM, Di Liberto G, Penin F, Kara R, Richard Y, Dussaix E, Feray C. Frequent compartmentalization of hepatitis C virus variants in circulating B cells and monocytes. Hepatology. 2004;**39**:817-825

[14] Blackard JT, Kemmer N, Sherman KE. Extrahepatic replication of HCV: Insights into clinical manifestations and biological consequences. Hepatology. 2006;**44**:15-22

[15] Pham TNQ, King D, MacParland SA, McGrath JS, Reddy SB, Bursey FR, Michalak TI. Hepatitis C virus replicates in the same immune cell subsets in chronic hepatitis C and occult infection. Gastroenterology. 2008;**134**:812-822

[16] Zignego AL, Craxi A. Extrahepatitc manifestations of hepatitis C virus infection. Clinics in Liver Disease. 2008;**12**:611-636

[17] Kondo Y, Shimosegawa T. Direct effects of hepatitis C virus on the lymphoid cells. World Journal of Gastroenterology. 2013;**19**:7889-7895

[18] Dai B, Chen AY, Corkum CP, Peroutka RJ, Landon A, Houng S, Muniandy PA, Zhang Y, Lehermann E, Mazan-Mamczarz K, Steinhardt J, Shlyak M, Chen QC, Becker KG, Livak F, Michalak TI, Talvani R, Gartenhaus RB. Hepatitis C virus upregulates B-cell receptor signaling: A novel mechanism for HCV-associated B-cell lymphoproliferative disorders. Oncogene. 2016;**35**:2979-2990

[19] Klenerman P, Zinkernagle RM. What can be learnt about human immunodeficiency virus infection from a study of lymphocytic chriomeningitis virus. Immunological Reviews. 1997;**159**:5-16

[20] Ciurea A, Klenerman P, Hunziker L, Horvath E, Odermatt B, Ochsenbein AF, Hengartner H, Zinkernagel RM. Persistence of lymphocytic choriomeningitis virus at very low levels in immune mice. Proceedings of the National Academy of Sciences of the United States of America. 1999;**96**:11964-11969

[21] Homann D, McGavern DB, Oldsone MBA. Visualizing the viral burden: Phenotypic and functional alterations of T cells and APCs during persistent infection. Journal of Immunology. 2004;**172**:6239-6250

[22] Sundaravaradan V, Saxena SK, Ramakrishnan R, Yedavalli VR, Harris DT, Ahmad N. Differential HIV-1 replication in neonatal and adult blood mononuclear cells is influenced at the level of HIV-1 gene expression. Proceedings of the National Academy of Sciences of the United States of America. 2006;**103**:11701-11706

[23] Zignego AL, Ferri C, Giannini C, La Civita L, Careccia G, Longombardo G, Bellesi G, Caracciolo F, Thiers V, Gentilini P. Hepatitis C virus infection in mixed cryoglobulinemia and B-cell non-Hodgkin's lymphoma: Evidence for a pathogenetic role. Archives of Virology. 1997;**142**:545-555

[24] Nieters A, Kallinowski B, Brennan P, Ott M, Maynadié M, Benavente Y, Foretova L, Cocco PL, Staines A, Vornanen M, Whitby D, Boffetta P, Becker N, De Sanjosé S. Hepatitis C and risk of lymphoma: Results of the European multicenter case-control study EPILYMPH. Gastroenterology. 2006;**131**:1879-1886

[25] de Sanjose S, Benavente Y, Vajdic CM, Engels EA, Morton LM, Bracci PM, Spinelli JJ, Zheng T, Zhang Y, Franceschi S, Talamini R, Holly EA, Grulich AE, Cerhan JR, Hartge P, Cozen W, Boffetta P, Brennan P, Maynadié M, Cocco P, Bosch R, Foretova L, Staines A, Becker N, Nieters A. Hepatitis C and non-Hodgkin lymphoma among 4784 cases and 6269 controls from the International Lymphoma Epidemiology Consortium. Clinical Gastroenterology and Hepatology. 2008;**6**:451-458

[26] Armand M, Besson C, Hermine O, Davi F. Hepatitis C virus-associated marginal zone lymphoma. Best Practice & Research Clinical Haematology. 2017;**30**:41-49

[27] Tasleem S, Sood GK. Hepatitis C associated B-cell non-Hodgkin lymphoma: clinical features and the role of antiviral therapy. Journal of Clinical and Translational Hepatology. 2015;**3**:134-139

[28] Arcaini L, Besson C, Frigeni M, Fontaine H, Goldaniga M, Casato M, Visentini M, Torres HA, Loustaud-Ratti V, Peveling-Oberhag J, Fabris P, Rossotti R, Zaja F, Rigacci L, Rattotti S, Bruno R, Merli M, Dorival C, Alric L, Jaccard A, Pol S, Carrat F, Ferretti VV, Visco C, Hermine O. Interferon-free antiviral treatment in B-cell lymphoproliferative disorders associated with hepatitis C virus infection. Blood. 2016;**28**:2527-2532

[29] Radkowski M, Laskus T. Persistence of hepatitis C virus after successful treatment of chronic hepatitis C: Is hepatitis C infection for life? Liver Transplantation. 2005;**11**:114-116

[30] Michalak TI, Pham TNQ, Mulrooney-Cousins PM. Molecular diagnosis of occult HCV and HBV infections. Future Virology. 2007;**2**:451-465

[31] Pham TNQ, Michalak TI. Occult persistence and lymphotropism of hepatitis C virus infection. World Journal of Gastroenterology. 2008;**14**:2789-2793

[32] Pham TNQ, Michalak TI. Hepatitis C virus in peripheral blood mononuclear cells of individuals with isolated anti-hepatitis C virus antibody reactivity. Hepatology. 2008;**48**:350-351

[33] Carreño V, Bartolomé J, Castillo I, Quiroga JA. New perspectives in occult hepatitis C virus infection. World Journal of Gastroenterology. 2012;**18**:2887-2894. DOI: 10.3748/wjg.v18.i23.2887

[34] Skardasi G, Chen AY, Michalak TI. Authentic patient-derived hepatitis C virus infects and productively replicates in primary $CD4^+$ and $CD8^+$ T lymphocytes *in vitro*. Journal of Virology. 2018;**92**:e01790-17

[35] MacParland SA, Pham TN, Gujar SA, Michalak TI. De novo infection and propagation of wild-type hepatitis C virus in human T lymphocytes in vitro. The Journal of General Virology. 2006;**87**:3577-3586

[36] Laporte J, Bain C, Maurel O, Inchauspe G, Agut H, Cahour A. Differential distribution and internal translation efficiency of hepatitis C virus quasispecies present in dendritic and liver cells. Blood. 2003;**101**:52-57

[37] Willems M, Peerlinck K, Moshage H, Deleu I, Van den Eynde C, Vermylen J, Yap SH. Hepatitis C virus-RNA in plasma and in peripheral blood mononuclear cells of haemophiliacs with chronic hepatitis C: Evidence for viral replication in peripheral blood mononuclear cells. Journal of Medical Virology. 1994;**42**:272-278

[38] Yun Z-B, Sönnerborg A, Weiland O. Hepatitis C virus replication in liver and peripheral blood mononuclear cells of interferon-treated and untreated patients with chronic hepatitis C. Scandinavian Journal of Gastroenterology. 1994;**29**:82-86

[39] Shimizu YK, Igarashi H, Kanematu T, Fujiwara K, Wong DC, Purcell RH, Yoshikura H. Sequence analysis of the hepatitis C virus genome recovered from serum, liver,

and peripheral blood mononuclear cells of infected chimpanzees. Journal of Virology. 1997;**71**:5769-5773

[40] Zignego AL, Giannini C, Monti M, Gragnani L, Hepatitis C. Virus lymphotropism: Lessons from a decade of studies. Digestive and Liver Disease. 2007;**39**(Suppl. 1):S38-S45

[41] Berthillon P, Inchauspé G, Trépo C. Evidence for lymphotropic strain of HCV. Hepatologia Clinica. 1998;**6**:71-75

[42] Navas S, Martin J, Quiroga JA, Castillo I, Carreño V. Genetic diversity and tissue compartmentalization of the hepatitis C virus genome in blood mononuclear cells, liver, and serum from chronic hepatitis C patients. Journal of Virology. 1998;**72**:1640-1646

[43] Radkowski M, Wang L-F, Vargas HE, Rakela J, Laskus T. Detection of hepatitis C virus replication in peripheral blood mononuclear cells after orthotopic liver transplantation. Transplantation. 1998;**66**:664-666

[44] Okuda M, Hino K, Korenaga M, Yamaguchi Y, Katoh Y, Okita K. Differences in hypervariable region 1 quasispecies of hepatitis C virus in human serum, peripheral blood mononuclear cells and liver. Hepatology. 1999;**29**:217-222

[45] Maggi F, Fornai C, Morrica A, Vatteroni ML, Giorgi M, Marchi S, Ciccorossi P, Bendinelli M, Pistello M. Divergent evolution of hepatitis C virus in liver and peripheral blood mononuclear cells of infected patients. Journal of Medical Virology. 1999;**57**:57-63

[46] Afonso AMR, Jiang J, Penin F, Tareau C, Samuel D, Petit MA, Bismuth H, Dussaix E, Féray C. Nonrandom distribution of hepatitis C virus quasispecies in plasma and peripheral blood mononuclear cell subsets. Journal of Virology. 1999;**73**:9213-9221

[47] Pal S, Sullivan DG, Kim S, Lai KK, Kae J, Cotler SJ, Carithers RL Jr, Wood BL, Perkins JD, Gretch DR. Productive replication of hepatitis C virus in peripheral lymph nodes in vivo: Implications of HCV lymphotropism. Gastroenterology. 2006;**130**:1107-1116

[48] Roque-Afonso AM, Ducoulombier D, Di Liberto G, Kara R, Gigou M, Dussaix E, Samuel D, Feray C. Compartmentalization of hepatitis C virus genotypes between plasma and peripheral blood mononuclear cells. Journal of Virology. 2005;**79**:6349-6357

[49] Vera-Otarola J, Barria MI, Leon U, Marsac D, Carvallo P, Soza A, Lopez-Lastra M. Hepatitis C virus quasispecies in plasma and peripheral blood mononuclear cells of treatment naive chronically infected patients. Journal of Viral Hepatitis. 2009;**16**:633-643

[50] Forton DM, Karayiannis P, Mahmud N, Taylor-Robinson SD, Thomas HC. Identification of unique hepatitis C virus quasispecies in the central nervous system and comparative analysis of internal translational efficiency of brain, liver, and serum variants. Journal of Virology. 2004;**78**:5170-5183

[51] Durand T, Di Liberto G, Colman H, Cammas A, Boni S, Marcellin P, Cahour A, Vagner S, Feray C. Occult infection of peripheral B cells by hepatitis C variants which have low translational efficiency in cultured hepatocytes. Gut. 2010;**59**:934-942

[52] Blackard JT, Smeaton L, Hiasa Y, Horiike N, Onji M, Jamieson DJ, Rodriguez I, Mayer KH, Chung RT. Detection of hepatitis C virus (HCV) in serum and peripheral-blood mononuclear cells from HCV-monoinfected and HIV/HCV-coinfected persons. The Journal of Infectious Diseases. 2005;**92**:258-265

[53] Navas MC, Fuchs A, Schvoerer E, Bohbot A, Aubertin AM, Stoll-Keller F. Dendritic cell susceptibility to hepatitis C virus genotype 1 infection. Journal of Medical Virology. 2002;**67**:152-161

[54] Goutagny N, Fatmi A, De Ledinghen V, Penin F, Couzigou P, Inchauspé G, Bain C. Evidence of viral replication in circulating dendritic cells during hepatitis C virus infection. The Journal of Infectious Diseases. 2003;**187**:1951-1958

[55] Pham TNQ, MacParland SA, Coffin CS, Lee SS, Bursey FR, Michalak TI. Mitogen-induced upregulation of hepatitis C virus expression in human lymphoid cells. The Journal of General Virology. 2005;**86**:657-666

[56] Bagaglio S, Cinque P, Racca S, Pedale R, Grasso MA, Lazzarin A, Morsica G. Hepatitis C virus populations in the plasma, peripheral blood mononuclear cells and cerebrospinal fluid of HIV/hepatitis C virus-co-infected patients. AIDS. 2005;**19**(Suppl 3):S151-S165

[57] Radkowski M, Wilkinson J, Nowicki M, Adair D, Vargas H, Ingui C, Rakela J, Laskus T. Search for hepatitis C virus negative-strand RNA sequences and analysis of viral sequences in the central nervous system: Evidence of replication. Journal of Virology. 2002;**76**:600-608

[58] Sansonno D, Lotesoriere C, Cornacchiulo V, Fanelli M, Gatti P, Iodice G, Racanelli V, Dammacco F. Hepatitis C virus infection involves CD34(+) hematopoietic progenitor cells in hepatitis C virus chronic carriers. Blood. 1998;**92**:3328-3337

[59] Sarhan MA, Chen AY, Russell RS, Michalak TI. Patient-derived hepatitis C virus and JFH-1 clones differ in their ability to infect human hepatoma cells and lymphocytes. The Journal of General Virology. 2012;**93**:2399-2407

[60] Marukian S, Jones CT, Andrus L, Evans MJ, Ritola KD, Charles ED, Rice CM, Dustin LB. Cell culture-produced hepatitis C virus does not infect peripheral blood mononuclear cells. Hepatology. 2008;**48**:1843-1850

[61] Quer J, Cos J, Murillo P, Esteban JI, Esteban R, Guardia J. Improved attachment of natural HCV isolate to Daudi cells upon elimination of immune complexes and close pH control. Intervirology. 2005;**48**:285-291

[62] Shimizu YK, Iwamoto A, Hijikata M, Purcell RH, Yoshikura H. Evidence for in vitro replication of hepatitis C virus genome in a human T-cell line. Proceedings of the National Academy of Sciences of the United States of America. 1992;**89**:5477-5481

[63] Sarhan MA, Pham TN, Chen AY, Michalak TI. Hepatitis C virus infection of human T lymphocytes is mediated by CD5. Journal of Virology. 2012;**86**:3723-3735

[64] Sarhan MA, Chen AY, Michalak TI. Differential expression of candidate virus receptors in human T lymphocytes prone or resistant to infection with patient-derived hepatitis C virus. PLoS One. 2013;**8**:e62159

[65] Shimizu YK, Purcell RH, Yoshikura H. Correlation between the infectivity of hepatitis C virus in vivo and its infectivity in vitro. Proceedings of the National Academy of Sciences of the United States of America. 1993;**90**:6037-6041

[66] Sung VM, Shimodaira S, Doughty AL, Picchio GR, Can H, Yen TS, Lindsay KL, Levine AM, Lai MM. Establishment of B-cell lymphoma cell lines persistently infected with hepatitis C virus in vivo and in vitro: The apoptotic effects of virus infection. Journal of Virology. 2003;**77**:2134-2146

[67] Kondo Y, Sung VM, Machida K, Liu M, Lai MM. Hepatitis C virus infects T cells and affects interferon-gamma signaling in T cell lines. Virology. 2007;**361**:161-173

[68] Kondo Y, Machida K, Liu HM, Ueno Y, Kobayashi K, Wakita T, Shimosegawa T, Lai MM. Hepatitis C virus infection of T cells inhibits proliferation and enhances Fas-mediated apoptosis by down-regulating the expression of CD44 splicing variant 6. The Journal of Infectious Diseases. 2009;**199**:726-736

[69] Morsica G, Tambussi G, Sitia G, Novati R, Lazzarin A, Lopalco L, Mukenge S. Replication of hepatitis C virus in B lymphocytes (CD19+). Blood. 1999;**94**:1138-1139

[70] Michalak TI. Immune cell reservoirs of persisting hepatitis C virus. Gut. 2010;**59**:867-868

[71] Laskus T, Radkowski M, Piasek A, Nowicki M, Horban A, Cianciara J, Rakela J. Hepatitis C virus in lymphoid cells of patients coinfected with human immunodeficiency virus type 1: evidence of active replication in monocytes/macrophages and lymphocytes. The Journal of Infectious Diseases. 2000;**181**:442-448

[72] MacParland SA, Chen AY, Corkum CP, Pham TN, Michalak TI. Patient-derived hepatitis C virus inhibits $CD4^+$ but not $CD8^+$ T lymphocyte proliferation in primary T cells. Virology Journal. 2015;**12**:93

[73] Lauer GM, Ouchi K, Chung RT, Nguyen TN, Day CL, Purkis DR, Reiser M, Kim AY, Lucas M, Klenerman P, Walker BD. Comprehensive analysis of CD8(+)-T-cell responses against hepatitis C virus reveals multiple unpredicted specificities. Journal of Virology. 2002;**76**:6104-6113

[74] Rehermann B. Hepatitis C virus versus innate and adaptive immune responses: A tale of coevolution and coexistence. The Journal of Clinical Investigation. 2009;**119**:1745-1754

[75] Ploss A, Evans MJ. Hepatitis C virus host cell entry. Current Opinion in Virology. 2012;**2**:14-19

[76] Bartosch B, Vitelli A, Granier C, Goujon C, Dubuisson J, Pascale S, Scarselli E, Cortese R, Nicosia A, Cosset FL. Cell entry of hepatitis C virus requires a set of co-receptors

that include the CD81 tetraspanin and the SR-B1 scavenger receptor. The Journal of Biological Chemistry. 2003;**278**:41624-41630

[77] Germi R, Crance J, Garin D, Guimet J, Lortat-Jacob H, Ruigrok RWH, Zarski J, Drouet E. Cellular glycosaminoglycans and low density lipoprotein receptor are involved in hepatitis C virus adsorption. Journal of Medical Virology. 2002;**68**:206-215

[78] Kapadia SB, Barth H, Baumert T, McKeating JA, Chisari FV. Initiation of hepatitis C virus infection is dependent on cholesterol and cooperativity between CD81 and scavenger receptor B type I. Journal of Virology. 2007;**81**:374-383

[79] Evans MJ, von Hahn T, Tscherne DM, Syder AJ, Panis M, Wolk B, Hatziioannou T, McKeating JA, Bieniasz PD, Rice CM. Claudin-1 is a hepatitis C virus co-receptor required for a late step in entry. Nature 2007;**446**:801-805

[80] Benedicto I, Molina-Jimenez F, Bartosch B, Cosset FL, Lavillette D, Prieto J, Moreno-Otero R, Valenzuela-Fernandez A, Aldabe R, Lopez-Cabrera M, Majano PL. The tight junction-associated protein occludin is required for a postbinding step in hepatitis C virus entry and infection. Journal of Virology. 2009;**83**:8012-8020

[81] Liu S, Yang W, Shen L, Turner JR, Coyne CB, Wang T. Tight junction proteins claudin-1 and occludin control hepatitis C virus entry and are downregulated during infection to prevent superinfection. Journal of Virology. 2009;**83**:2011-2014

[82] Ploss A, Evans MJ, Gaysinskaya VA, Panis MH, de Jong YP, Rice CM. Human occludin is a hepatitis C virus entry factor required for infection of mouse cells. Nature. 2009;**457**:882-886

[83] Lupberger J, Zeisel M, Xiao F, Thumann C, Fofana I, Zona L, Davis C, Mee CJ, Turek M, Gorke S, Royer C, Fischer B, Zahid MN, Lavillette D, Fresquet J, Cosset FL, Rothenberg SM, Pietschmann T, Patel AH, Pessaux P, Doffoël M, Raffelsberger W, Poch O, McKeating JA, Brino L, Baumert TF. EGFR and EphA2 are host factors for hepatitis C virus entry and possible targets for antiviral therapy. Nature Medicine. 2011;**17**:589-596

[84] Sainz B Jr, Barretto N, Martin DN, Hiraga N, Imamura M, Hussain S, Marsh KA, Yu X, Chayama K, Alrefai WA, Uprichard SL. Identification of the Niemann-Pick C1-like 1 cholesterol absorption receptor as a new hepatitis C virus entry factor. Nature Medicine. 2012;**18**:281-285

[85] Martin DN, Uprichard SL. Identification of transferrin receptor 1 as a hepatitis C virus entry factor. Proceedings of the National Academy of Sciences of the United States of America. 2013;**110**:10777-10782

[86] Douam F, Bobay LM, Maurin G, Fresquet J, Calland N, Maisse C, Durand T, Cosset FL, Féray C, Lavillette D. Specialization of hepatitis C virus envelope glycoproteins for B lymphocytes in chronically infected patients. Journal of Virology. 2015;**90**:992-1008

[87] Chen CL, Huang JY, Wang CH, Tahara SM, Zhou L, Kondo Y, Schechter J, Su L, Lai MM, Wakita T, Cosset FL, Jung JU, Machida K. Hepatitis C virus has a genetically determined lymphotropism through co-receptor B7.2. Nature Communications. 2017;**8**:13882

[88] Klatzmann D, Champagne E, Chamaret S, Gruest J, Guetard D, Hercend T, Gluckman JC, Montagnier L. HIV T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. Nature. 1984;**312**:767-768

[89] Zaitseva M, Blauvelt A, Lee S, Lapham CK, Klaus-Kovtun V, Mostowski H, Manischewitz J, Golding H. Expression and function of CCR5 and CXCR4 on human Langerhans cells and macrophages: implications for HIV primary infection. Nature Medicine. 1997;**3**:1369-1375

[90] Tatsuo H, Ono N, Tanaka K, Yanagi Y. SLAM (CDw150) is a cellular receptor for measles virus. Nature. 2000;**406**:893-897

[91] Sakaguchi M, Yoshikawa Y, Yamanouchi K. Growth of measles virus in epithelial and lymphoid tissue of cynomologous monkey. Microbiology and Immunology. 1986;**30**:883-891

[92] Nemerow GR, Siaw MF, Cooper NR. Purification of Epstein-Barr virus/C3d complement receptor of human B lymphocytes: Antigenic and functional properties of the purified protein. Journal of Virology. 1986;**58**:709-712

[93] Kasai K, Sato Y, Kameya T, Inoue H, Yoshimura H, Kon S, Kikuchi K. Incidence of latent infection of Epstein-Barr virus in lung cancers—An anlaysis of EBER1 expression in lung cancers by in situ hybridization. The Journal of Pathology. 1994;**174**:257-265

[94] Lee J, Kuchen S, Fischer R, Chang S, Lipsky PE. Identification and characterization of a human CD5+ pre-naive B cell population. Journal of Immunology. 2009;**182**:4116-4126

[95] Su WC, Machida K, Lai MMC. Extrahepatic replication of HCV. In: Muyamura T, Lemon SM, Walker CM, Wakita T, editors. Hepatitis C Virus II. Springer Japan. 2016. pp. 165-184

[96] Kondo Y, Ueno Y, Kakazu E, Kobayashi K, Shiina M, Tamai K, Machida K, Inoue J, Wakui Y, Fukushima K, Obara N, Kimura O, Shimosegawa T. Lymphotropic HCV strain can infect human primary naïve $CD4^+$ cells and affect their proliferation and IFN-γ secretion activity. Journal of Gastroenterology. 2011;**46**:232-241

[97] Bergqvist A, Rice CM. Transcriptional activation of the interleukin-2 promoter by hepatitis C virus core protein. Journal of Virology. 2001;**75**:772-781

[98] Bergqvist A, Sundström S, Dimberg LY, Gylfe E, Masucci MG. The hepatitis C virus core protein modulates T cell responses by inducing spontaneous and altering T-cell receptor-triggered Ca2+ oscillations. The Journal of Biological Chemistry. 2003;**278**:18877-18883

[99] Domínguez-Villar M, Muñoz-Suano A, Anaya-Baz B, Aguilar S, Novalbos JP, Giron JA, Rodríguez-Iglesias M, Garcia-Cozar F. Hepatitis C virus core protein up-regulates anergy-related genes and a new set of genes, which affects T cell homeostasis. Journal of Leukocyte Biology. 2007;**82**:1301-1310

[100] Yao ZQ, King E, Prayther D, Yin D, Moorman J. T cell dysfunction by hepatitis C virus core protein involves PD-1/PDL-1 signaling. Viral Immunology. 2007;**20**:276-287

[101] Bhattarai N, McLinden JH, Xiang J, Kaufman TM, Stapleton JT. Conserved motifs within hepatitis C virus envelope (E2) RNA and protein independently inhibit T cell activation. PLoS Pathogens. 2015;**11**:e1005183

[102] Bhattarai N, McLinden JH, Xiang J, Mathahs MM, Schmidt WN, Kaufman TM, Stapleton JT. Hepatitis C virus infection inhibits a Src-kinase regulatory phosphatase and reduces T cell activation in vivo. PLoS Pathogens. 2017;**13**:e1006232

[103] Kasama Y, Sekiguchi S, Saito M, Tanaka K, Satoh M, Kuwahara K, Sakaguchi N, Takeya M, Hiasa Y, Kohara M, Tsukiyama-Kohara K. Persistent expression of the full genome of hepatitis C virus in B cells induces spontaneous development of B-cell lymphomas in vivo. Blood. 2010;**116**:4926-4933

[104] Monti G, Pioltelli P, Saccardo F, Campanini M, Candela M, Cavallero G, De Vita S, Ferri C, Mazzaro C, Migliaresi S, Ossi E, Pietrogrande M, Gabrielli A, Galli M, Invernizzi F. Incidence and characteristics of non-Hodgkin lymphomas in a multicenter case file of patients with hepatitis C virus-related symptomatic mixed cryoglobulinemias. Archives of Internal Medicine. 2005;**165**:101-105

[105] Young RM, Staudt LM. Targeting pathological B cell receptor signalling in lymphoid malignancies. Nature Reviews. Drug Discovery. 2013;**12**:229-243

[106] Rosa D, Saletti G, De Gregorio E, Zorat F, Comar C, D'Oro U, Nuti S, Houghton M, Barnaba V, Pozzato G, Abrignani S. Activation of naïve B lymphocytes via CD81, a pathogenetic mechanism for hepatitis C virus-associated B lymphocyte disorders. Proceedings of the National Academy of Sciences of the United States of America. 2005;**102**:18544-18549

[107] Feldmann G, Nischalke HD, Nattermann J, Banas B, Berg T, Teschendorf C, Schmiegel W, Dührsen U, Halangk J, Iwan A, Sauerbruch T, Caselmann WH, Spengler U. Induction of interleukin-6 by hepatitis C virus core protein in hepatitis C-associated mixed cryoglobulinemia and B-cell non-Hodgkin's lymphoma. Clinical Cancer Research. 2006;**12**:4491-4498

[108] Mazan-Mamczarz K, Hagner PR, Corl S, Srikantan S, Wood WH, Becker KG, Gorospe M, Keene JD, Levenson AS, Gartenhaus RB. Post-transcriptional gene regulation by HuR promotes a more tumorigenic phenotype. Oncogene. 2008;**27**:6151-6163

[109] Machida K, Cheng KT, Sung VM, Shimodaira S, Lindsay KL, Levine AM, Lai MY, Lai MM. Hepatitis C virus induces a mutator phenotype: enhanced mutations of immunoglobulin and protooncogenes. Proceedings of the National Academy of Sciences of the United States of America. 2004;**101**:4262-4267

[110] Dammacco F, editor. HCV infection and cryoglobulinemia. Italia: Springler-Verlang; 2012

[111] Tucci F, Küppers R. Role of hepatitis C virus in B cell lymphoproliferation. Virologica Sinica. 2014;**29**:3-6

Chapter 4

The Molecular Background Associated with the Progression of Hepatitis C to Hepatocellular Carcinoma

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Additional information is available at the end of the chapter

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Abstract

Hepatocellular carcinoma (HCC) is a major health problem worldwide. The DNA PM of cancer-related genes plays an important role in the development and progression of HCC. The data reported in our studies provide evidence that PM of p73, p14, and O6-MGMT is associated with HCC, whereas PM of the APC gene is more common in chronic hepatitis (CH) cases. Thus, it could be used as a maker for early detection of HCV-induced chronic active hepatitis. A panel of four genes APC, p73, p14, and O6-MGMT independently affected the classification of cases into HCC and CH with accuracy (89.9%), sensitivity (83.9%), and specificity (94.7%). Also, the detection of PM of APC, FHIT, p15, p16, and E-cadherin in peripheral blood of HCV-infected patients is a highly sensitive and specific. Therefore, blood could be used as efficiently as tissue biopsies to assess PM of different genes. This could help in the follow-up of CH patients and early detection of HCC. We did not observe a significant difference in the methylation status according to the virus type HBV versus HCV. So, plasma DNA is a reliable resource for methylation studies in the future, irrespective of the type of hepatitis infection.

Keywords: hepatitis C virus-genotype 4, chronic hepatitis, hepatocellular carcinoma, promoter methylation

1. Introduction

Hepatocellular carcinoma (HCC) is a major health problem and it is the third most common cause of cancer-related death worldwide [1]. In Egypt HCC ranks the first in males and the second in females after breast cancer. It accounted for 33.6% in males and 13.5%

in females [2]. This high incidence of HCC is attributed to the high prevalence of HCV infection, especially genotype 4 in Egypt [3]. HCV infection has an estimated global prevalence of 2.5%, causing chronic liver disease in about 170 million people worldwide [4]. Although it has been estimated that 80% of HCC occurs in cirrhotic livers, the underlying molecular mechanisms of virus associated hepatocarcinogenesis are still unclear [5]. It has been suggested that HCV-encoded proteins may contribute to tumor progression through their direct and indirect interactions with host hepatic cells. Additionally, the generated status of chronic HCV inflammation is accompanied by immune-mediated destruction of infected hepatocytes, oxidative stress, virus-induced apoptosis and DNA damage leading to genomic instability and continuous regeneration that may be incorporated in liver cancer development [6].

Previous studies demonstrated that DNA methylation has a major role in the initiation and progression of various types of human cancers [7, 8]. Aberrant promoter methylation of tumor suppressor genes (TSGs), such as P14 or O6-methylguanine-DNA methyltransferase (O6MGMT) has been reported in relation to HCC development [9].

The term DNA methylation refers to the addition of a methyl group to the cytosine residue in the CpG islands. Normally, CpG islands are not methylated regardless of their transcriptional status, and methylation of the promoter regions of tumor suppressor genes (TSGs) or growth regulatory genes resulted in silencing of those genes, and cancer development. Since it was proven that different types of cancer showed distinct DNA methylation profiles, thus it could be possible to develop specific methylation signatures for those types of cancer [10].

The power of PM as a molecular marker is the ability of detecting its presence in a variety of sample types including fresh specimens, body fluids and archival paraffin-embedded tissues, as well as to the defined localization of the lesion in the CpG islands of the genes. Promoter methylation could be an important early event in the cascade of carcinogenesis and it can also be of important as prognostic and predictive marker [11]. The DNA methylation profiles in HCV-infected patients from Egypt have not been well studied yet, although it has the highest prevalence of HCV infection worldwide with approximately 14% of the population infected [12].

2. Concordance between tissue and plasma DNA methylation in HCC patients

Owing to the crucial effects of DNA promotor methylation in the development and progression of HCC, we investigated the role of DNA methylation events in the tissues of HCC patients for using five tumor suppressor genes: APC, FHIT, p15, p16, and E-cadherin. We also assessed the DNA methylation patterns of these genes in the plasma from the same patients and compared the tissue and plasma patterns [13]. This was done to investigate the concordance between tissue and plasma methylation patterns in Egyptian patients with HCV and/or HBV- associated HCC. Although liver biopsy is the current gold standard for detecting methylation events, imaging techniques are usually sufficient for liver cancer diagnosis and

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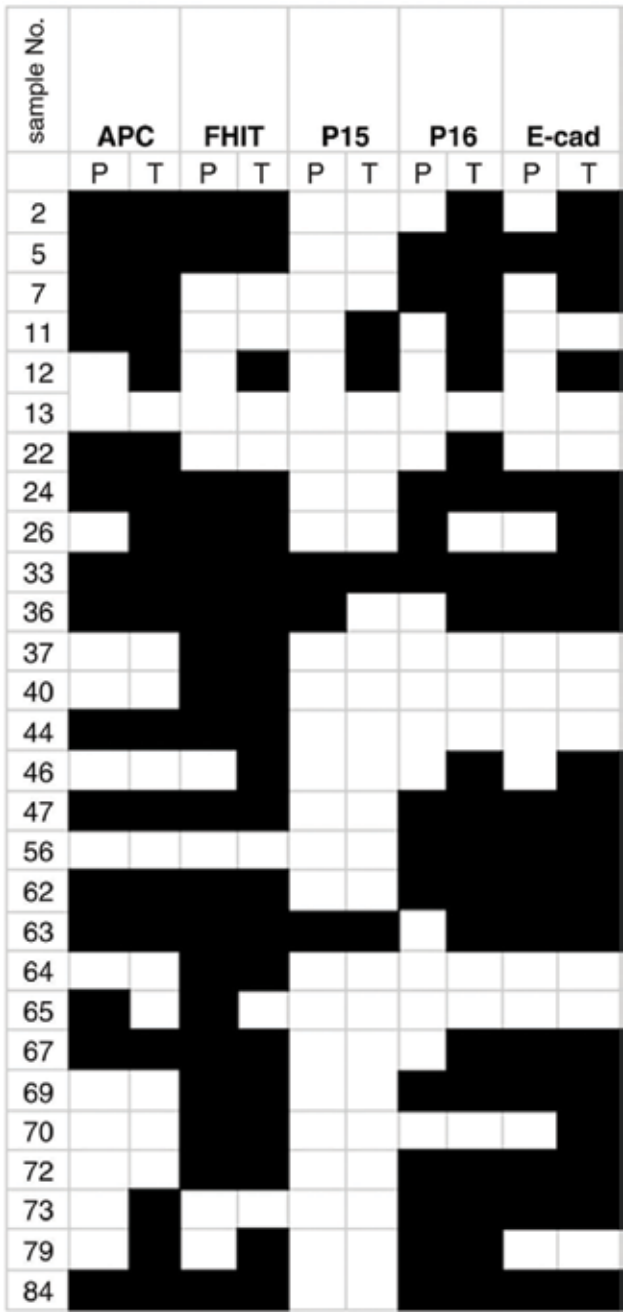


Figure 1. Summary of methylation analysis of APC, FHIT, p15, p16, and E-cadherin in 28 HCC samples and the corresponding plasma. Filled boxes indicate the presence of methylation and open boxes indicate the absence of methylation. T, tumor tissue; P, plasma.

therefore the need of tissue biopsy decreased markedly [14]. So it was essential to search for another tool for detection of promotor methylation in HCC by a simpler, easy and reliable technique.

Genes	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	*p*-Value
APC	77.8	90.0	93.3	69.2	0.001*
FHIT	85.7	85.7	90.5	66.7	0.001*
p15	50.0	95.8	66.7	92.0	0.045*
p16	60.0	87.5	92.3	46.7	0.037*
E-cadherin	68.4	100.0	100.0	60.0	0.0008*

PPV, positive predictive value; NPV, negative predictive value.*The concordance was significant for all five genes.

Table 1. The concordance between plasma DNA and tissue DNA.

We collected paired blood and tissue samples from 28 HCV and/or HBV- associated HCC patients from Egypt. DNA was extracted from those patients (tissue and blood) and the promotor methylation for ***APC, FHIT, p15, p16, and E-cadherin*** tumor suppressor genes were assessed using the EZ DNA Methylation-Direct TM Kit according to manufacturer's protocol.

We reported a statistically significant concordance between plasma and tissue methylation profiles [13]. The frequency of promoter methylation in tissue and plasma samples for the five tumor suppressor genes was as follows: ***APC*** promoter methylation was accounted for approximately 64.2% for tissue (18/28), and 53.5% for plasma (15/28), ***FHIT*** promoter methylation was accounted for 75.0% for tissue (21/28), and 67.8% for plasma (19/28), ***p15*** promoter methylation was accounted for 14.2% for tissue (4/28), and 10.7% for plasma (3/28), ***p16*** promoter methylation was accounted for approximately 71.4% for tissue (20/28) and 46.4% for plasma (13/28), and ***E-cadherin*** promoter methylation was accounted for 67.8% for tissue (19/28) and 46.4% for plasma (13/28) (**Figure 1**).

Although detection of promoter methylation in the plasma DNA was highly specific, it was not as sensitive for the matching change in tissue DNA, suggesting that DNA promotor methylation in tissues might originate in tumor cells before appearing in the vascular spaces (blood or plasma). The positive predictive value (PPV) was higher than the negative predictive value (NPV) for ***APC, FHIT, p16,*** and ***E-cadherin*** whereas, the negative predictive value was higher for ***p15*** (**Table 1**). Therefore, a previous study by Huang et al., [15] concluded that it may be useful to combine the plasma DNA methylation status of ***ELF, RASSF1A, p16***, and ***GSTP1*** with serum AFP for HCC screening and several studies had confirmed these data [16, 17]. However controversial results were reported by Chang et al. [18] who found no agreement between plasma and tissue DNA samples. One possible explanation for the controversy in the results between the previously mentioned studies could be the small sample size in the study of Chang et al. (eight HCC patients only) and/or the use of RT-PCR which causes DNA degradation during amplification.

3. Methylation profile and viral status

Another interesting finding observed is that, there was no significant correlation between HBV or HCV infection and the incidence of promoter methylation, to suggest whether the viral status could be used to predict methylation and subsequent gene silencing for the five

	HBV	HCV	HBV infection type*
APC	0.107	0.634	0.508
FHIT	0.545	1	0.508
p15	0.481	1	0.288
p16	0.295	0.639	1
E-cadherin	0.273	0.629	0.66

The methylation profile was not significantly associated with the HBV, HCV, or *HBV Infection type: past infection or immune.

Table 2. Statistical association of hepatitis viral status and promoter methylation.

aforementioned tumor suppressor genes [13]. Therefore, plasma DNA could be used as a reliable source for methylation detection in HCC patients irrespective of the type of hepatitis viral infection (**Table 2**).

4. Increasing DNA promoter methylation is associated with disease progression from chronic hepatitis C to cirrhosis and hepatocellular carcinoma

As a continuation of our previous studies, which showed a concordance between tissue and plasma DNA methylation, and hence the validity of using plasma DNA methylation profile as a marker for HCC [13], we had assessed the methylation frequency of three tumor suppressor genes (***P14, P15***, ***P73***) and a mismatch repair gene ***(O6MGMT)*** in the plasma of 516 Egyptian patients with HCV-related liver disease, during the period from 2010 to 2012, to identify candidate epigenetic biomarkers for prediction of HCC [19]. Subjects were divided into 4 clinically well-defined groups as follow: the HCC group (n = 208), liver cirrhosis group (LC; n = 108), chronic hepatitis C group (CH; n = 100), and normal control group (NC; n = 100). The methylation status of the target genes was analyzed in patients' plasma using EpiTect Methyl qPCR Array technology. According to the manufacturer's instructions, the four studied genes ***(P14, P15, P73 and O6MGMT)*** were considered methylated if >10% and intermediately methylated if >60%.

We found significant differences in the frequency of PM of all studied genes within the different stages of chronic liver disease and HCC (**Table 3** and **Figure 2**). The methylation frequency of ***P14*** gene was 48.1% (100/208) in HCC, 48.1% (52/108) in LC, 16% (16/100) in CH and 8% (8/100) in NC. Out of the studied patients 32/208 (15.4%), 16/108 (14.8%) and 8/100 (8%) were intermediately methylated in HCC, LC and chronic hepatitis C groups respectively, with a statistically significant difference between the studied groups (p = 0.008). Accordingly, p14 is preferentially methylated in HCV related HCC [20].

As for ***p15***, the methylation frequency was 44.2% (92/208) in HCC, 33.3% (36/108) in LC, 20% (20/100) in CH and 4% (4/100) in NC. While intermediate methylation was found in 32/208 (15.4%) of HCC, 20/108 (18.5%) in LC, in 8/100 (8%) CH and 4/100 (4%) in NC with a statistically significant difference between the studied groups (p = 0.006).

Gene	HCC	HCV with liver cirrhosis	Chronic Hepatitis C	Control	p value
	n = 208(%)	n = 108(%)	n = 100(%)	n = 100(%)	
P14	M 100 (48.1)	52 (48.1)	16 (16)	8 (8)	0.008
	U 108 (51.9)	56 (51.9)	84 (84)	92 (92)	
P15	M 92 (44.2)	36 (33.3)	20 (20)	4 (4)	0.006
	U 116 (55.8)	72 (66.7)	80 (80)	96 (96)	
O6MGMT	M 84 (40.4)	60 (55.6)	20 (20)	4 (4)	<0.001
	U 124 (59.6)	48 (44.4)	80 (80)	96 (96)	
P73	M 136 (65.4)	72 (66.7)	32 (32)	4 (4)	<0.001
	U 72 (34.6)	36 (33.3)	68 (68)	96 (96)	

Methylated (M); Unmethylated (U).

Table 3. Methylation frequency of P14, P15, O6MGMT and P73 genes in different studied groups.

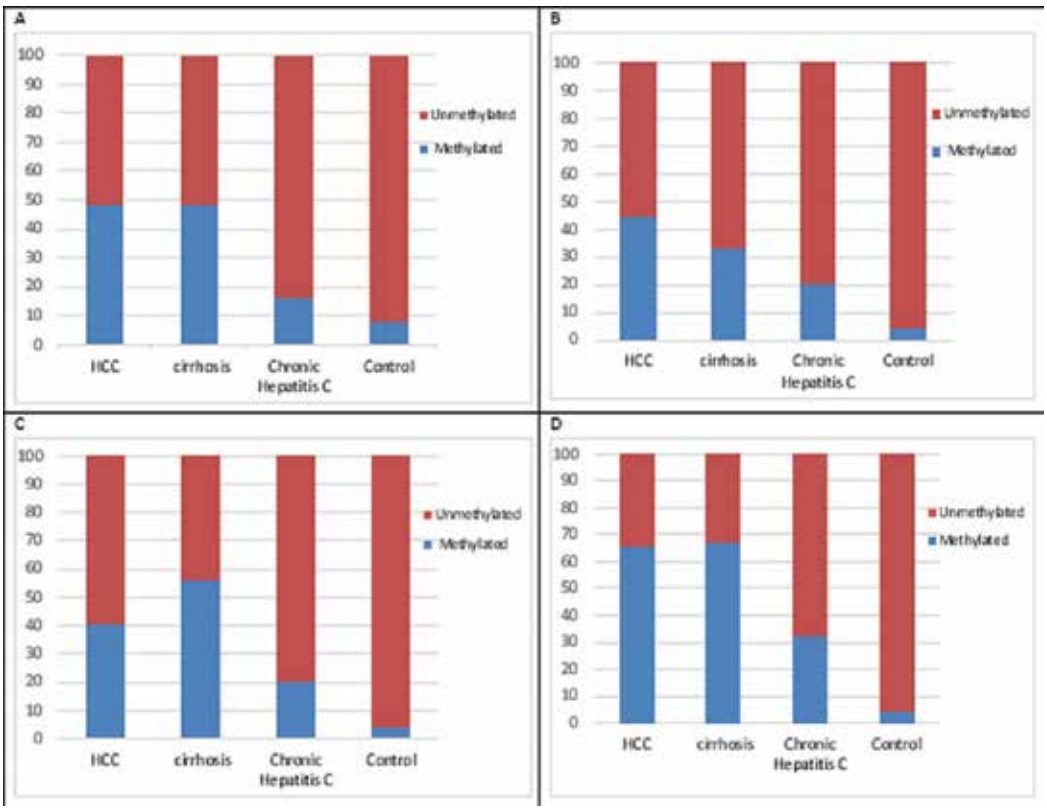


Figure 2. Methylation frequency of (A) P14 gene; (B) P15 gene; (C) O6MGMT gene; and (D) P73 gene in the studied groups.

The methylation frequency of ***O6MGMT*** gene was 40.4% (84/208) in HCC, 55.6% (60/108) in LC, 20% (20/100) in CH and 4% (4/100) in NC. While intermediately methylated in 48/208 (23.1%) in HCC and 36/108 (33.3%) in LC, with a statistically significant difference between the studied groups (p value<0.001).

The methylation frequency of ***P73*** gene was detected in 65.4% (136/208) in HCC, 66.7% (72/108) in LC, 32% (32/100) in CH and 4% (4/100) in NC. While intermediate methylation was found in 88/208 (42.3%) in HCC, 56/108 (51.9%) in LC, 24/100 (24%) in CH and 4/100 (4%) in NC, with a statistically significant difference between the studied groups (p value<0.001). Statistically significant differences were reported among the four studied groups regarding the PM of all studied genes (**Table 4**).

Thus, it could be concluded that, the methylation frequency increases with the progression of liver disease and thus it that could be used to monitor whether a patient with chronic hepatitis C is likely to progress to liver cirrhosis or even HCC or not. Moreover, the process of PM does not represent an early event in hepatocarcinogenesis cascade but it increases and continues with disease progression to cancer.

Based on our data regarding the high methylation frequency of ***APC, FHIT, CDH1 and p16*** in the plasma and tissues of HBV and HCV-associated HCC patients from Egypt [13] we sought to confirm this data in a larger cohort of HCV-genotype-4 infected patients using a larger panel of 11 genes (***p14, p15, p16, p73, APC, FHIT, DAPK1, CDH1, RARb, RASSF1A, and O6MGMT***). The newly tested group included (1) asymptomatic carriers, (2) CH patients with cirrhosis and (3) HCC. PM of the 11 genes were assessed in the Peripheral Blood Lymphocytes (PBLs) and the tissues of 31 HCC with their adjacent normal tissue (ANT), 38 CH and 13 normal hepatic tissue (NHT); which represents the progression from NHT to HCC in the HCV genotype 4-infected persons [21]. Promotor methylation of these genes was assessed by methylation-specific PCR (MSP). APC and O6-MGMT protein expression was assessed by immunohistochemistry (IHC) in the studied HCC and CH tissue samples.

	HCC	**Cirrhosis**	**Chronic hepatitis C**	**Control**
P14	HCC	0.954[a]	0.035[b]	0.004[c]
	Cirrhosis		0.050[d]	0.004[e]
	Chronic C			0.546[f]
	Control			
P15	HCC	0.409[a]	0.090[b]	<0.001[c]
	Cirrhosis		0.554[d]	0.024[e]
	Chronic C			0.223[f]
	Control			
MGMT	HCC	0.328[a]	0.016[b]	0.003[c]
	Cirrhosis		0.002[d]	<0.001[e]
	Chronic C			0.189[f]
	Control			
TP73	HCC	0.858[a]	0.037[b]	<0.001[c]
	Cirrhosis		0.058[d]	<0.001[e]
	Chronic C			0.026[f]
	Control			

Table 4. Pairwise comparison among the studied groups.

5. Analysis of DNA methylation events of the 11 tested genes among the studied groups

A high methylation frequency was reported for all studied genes (except for p15) in the PBL and tissues with increasing methylation index as the disease progresses (**Figure 3**). The PM of the 11 tested genes assessed in 13 NHT samples showed no methylation events in ***p15, p73, RARb, RASS, F1A*** or ***O6MGMT***. However ***p14 PM*** was estimated in 46.2% of the cases followed by ***APC*** which was methylated in 30.8% of the cases. There was a significant difference in MF between NHT and CH groups regarding ***APC, FHIT, DAPK*** and ***RASSF*** genes. Also MF of ***p14, p73, RASSF1A, CDH1*** and ***O6MGMT*** was significantly higher in HCC and their ANT. However MF of ***APC*** was higher in CH (**Figure 4** and **Table 5**). Among the four groups enrolled (HCC, CH, ASC, NHT) binary logistic regression in PROC LOGISTIC for each gene was used. Our results indicate that there is a significant interaction between disease state (different groups) and DNA methylation of the tested genes (**Figure 5a–k**). As shown in **Figure 6**, there is a significant group effect for ***APC*** (ASC group is different from HCC Group, $p = 0.0006$). This interaction is explained by the fact that there is a bigger difference between methylation and un-methylation for the CH group compared to any other group, especially the NHT. For ***DAPK1*** (**Figure 5g**), there is a marginal group effect, not significant by our corrected level of

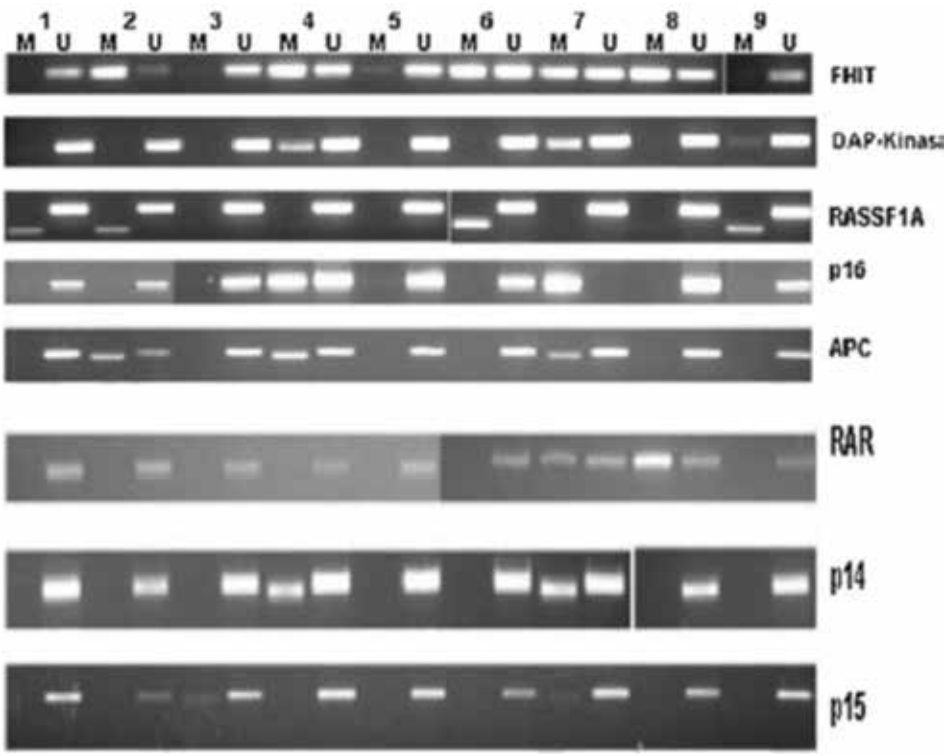
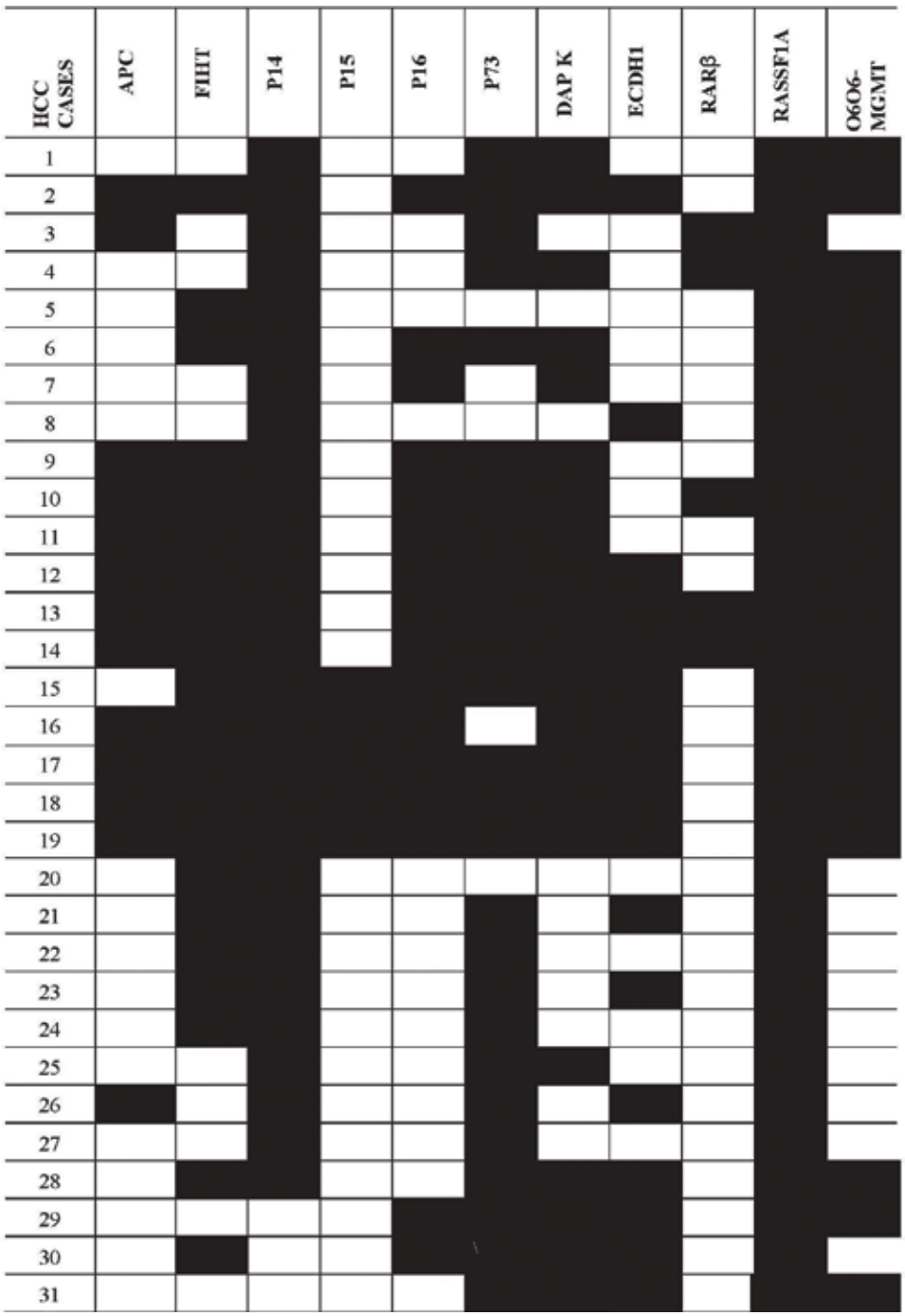


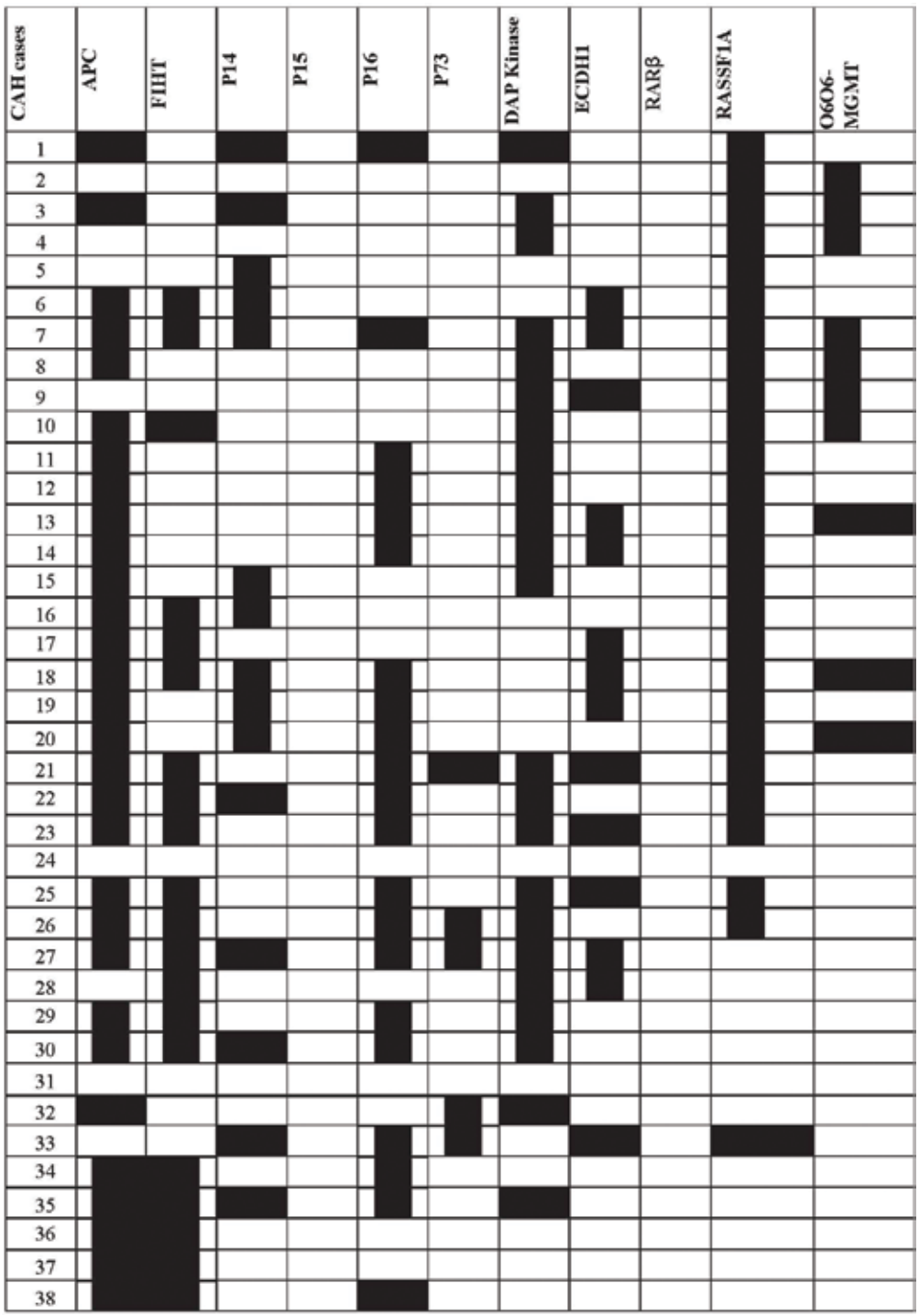
Figure 3. Methylation-specific PCR analyses of nine representative HCC samples (labeled 1–9 on the top). Each gene is indicated on the right. Both methylated (M) and unmethylated (U) reactions were amplified for each bisulfite-treated DNA and run in a 4% agarose gel.

P = 0.004 (NHT is significantly different from HCC P = 0.007) and ***RARb*** (**Figure 5h**) (NHT is different from-HCC Group P = 0.007). In contrast, there were significant methylation effects for ***APC*** p < 0.0001), ***FHIT*** (P < 0.0001), ***p15***, (P = 0.003), ***p14*** (P < 0.0001), ***DAPK1*** (P < 0.0001), ***RARb*** (P < 0.0001) and ***E-cadherin*** (P < 0.0001).

(A)



(B)


CAH cases
APC
FIIIT
P14
P15
P16
P73
DAP Kinase
ECDH1
RARβ
RASSF1A
O6O6-MGMT
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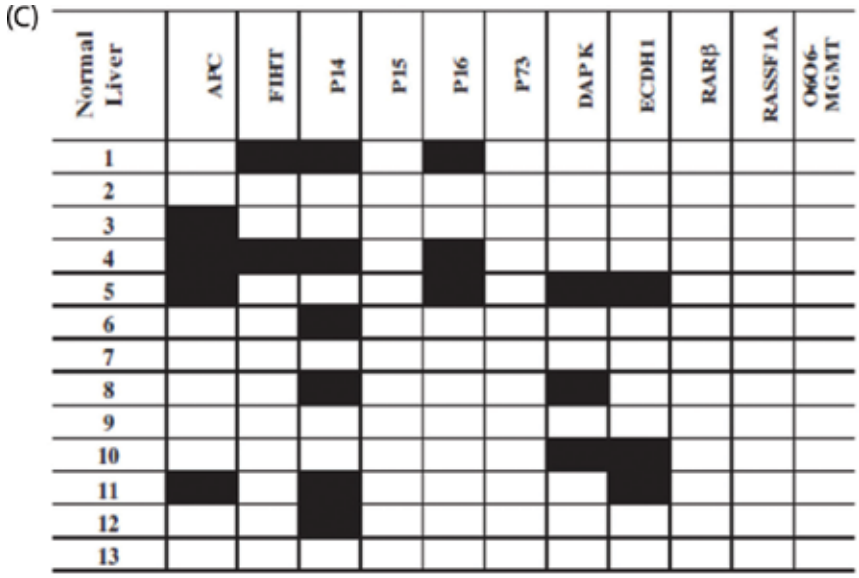

Figure 4. (A) Methylation of 11 genes in hepatocellular carcinoma patients; (B) Methylation of 11 genes in patients with chronic liver diseases; (C) Methylation of 11 genes in normal liver individuals. Dark squares depict methylation and blank squares depict unmethylation.

Genes	Normal liver N = 13 (%)	Chronic hepatitis (CH)		Hepatocellular carcinoma HCC		p-Value*	
		(Tissue) (38) (%)	(PBL) (20) (%)	(HCC) (31) (%)	(ANT) (31) (%)	(CH and HCC)	(CH and ANT)
APC	4 (30.8)	33 (86.8)	16 (80)	13 (41.9)	14 (45.2)	<0.001	<0.001
FHIT	2 (15.4)	20 (52.6)	6 (30)	21 (67.7)	20 (64.5)	0.204	0.005
P15	0 (0)	0 (0)	0 (0)	5 (16.1)	5 (16.1)	0.010	#
P73	0 (0)	8 (21.1)	1 (5.0)	26 (83.9)	23 (74.2)	<0.001	<0.001
P14	6 (46.2)	17 (44.7)	10 (50)	28 (90.3)	28 (90.3)	<0.001	<0.001
P16	3 (23.1)	15 (39.5)	9 (45)	14 (45.2)	19 (61.3)	0.634	0.390
DAPK	3 (23.1)	22 (57.9)	12 (60)	21 (67.7)	22 (71)	0.401	0.023
RARb	0 (0)	0 (00)	0 (0)	5 (16.1)	3 (9.7)	0.015	#
RASSF	0 (0)	26 (68.4)	20 (100)	31 (100)	31 (100)	0.001	<0.001
O6O6-MGMT	0 (0)	10 (26.3)	10 (50.0)	21 (67.7)	20 (64.5)	<0.001	<0.001
CDH1	3 (23.1)	7 (18.4)	8 (40.0)	17 (54.8)	14 (45.2)	0.002	0.004

*p-Values <0.05 are considered significant. #Numbers are too small for a valid statistical analysis.

Table 5. Methylation profile of the 11 genes in CH, HCC and normal liver tissues.

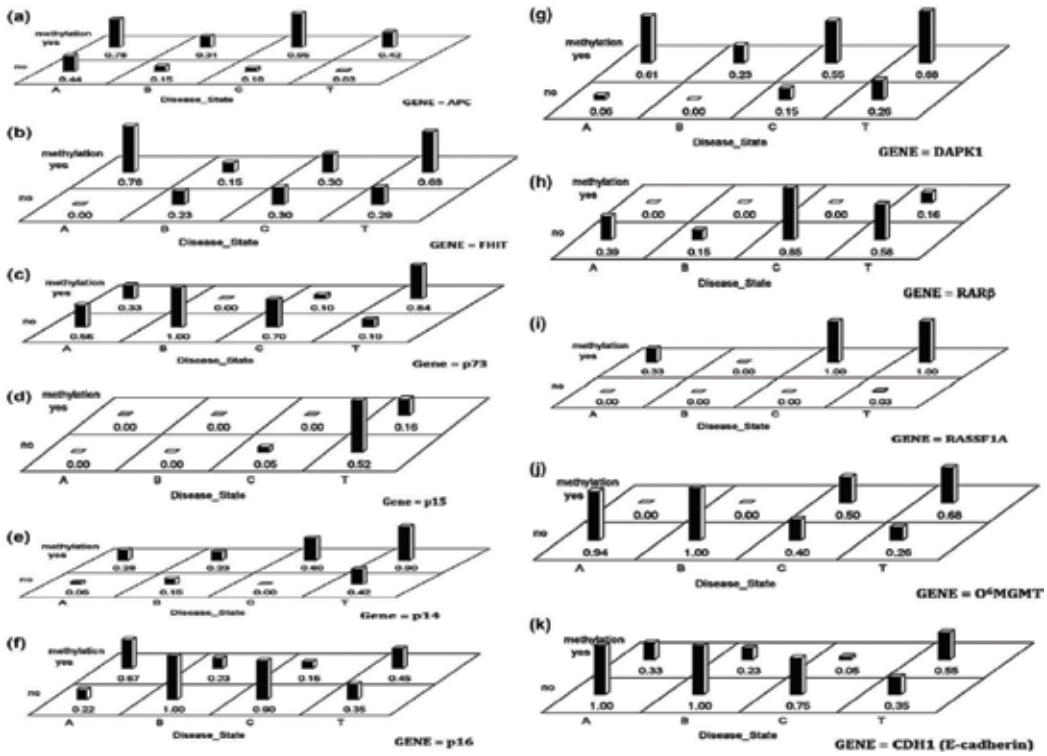

Figure 5. Differences in the methylation frequency among the four studied groups. (T = HCC, C = CAH with cirrhosis, A = asymptomatic carrier and B = normal hepatic tissue).

Our data regarding the ***p15*** gene confirms our previous study in which ***p15*** methylation was reported in 14.2% only of HCC cases [13, 19]. Within the studied groups, the methylation frequency of ***p14, p73, RASSF1A*** and ***O6MGMT*** was significantly higher in HCC and their ANT compared to CH and the NHT samples, whereas PM of ***APC*** was significantly higher in CH patients compared to all other groups. This was applied to PBL and tissues except for ***RASSF1A*** and ***O6MGMT*** in which the difference in the MF in PBL was statistically insignificant (**Figure 7).**

RASSF1A is a candidate TSG, which frequently shows PM and loss of heterozygosity (LOH) with consequent gene silencing in several human cancers [22]. The high MF reported here confirms the results of some recent studies including those of Qu and Lia [23, 24] who found PM of ***RASSF1A*** gene in 78 and 95% of HCC cases assessed. In our study, ***RASSF1A*** methylation was detected in all HCC cases and in 68.4% of CH cases (being second only to ***APC***). This finding is consistent with Araújo and Gioia et al. [25, 26] who reported an increase in ***RASSF1A*** PM with progression from regenerative conditions (e.g. cirrhosis) to hepatocellular nodules and HCC, as well as with Huang et al. [15] and Chan et al. [27] who reported ***RASSF1A*** methylation in the blood and tissues of HCC patients. Our results also showed an increasing frequency of ***p16*** PM from NHT to HCC which is in agreement with the earlier studies [28, 29].

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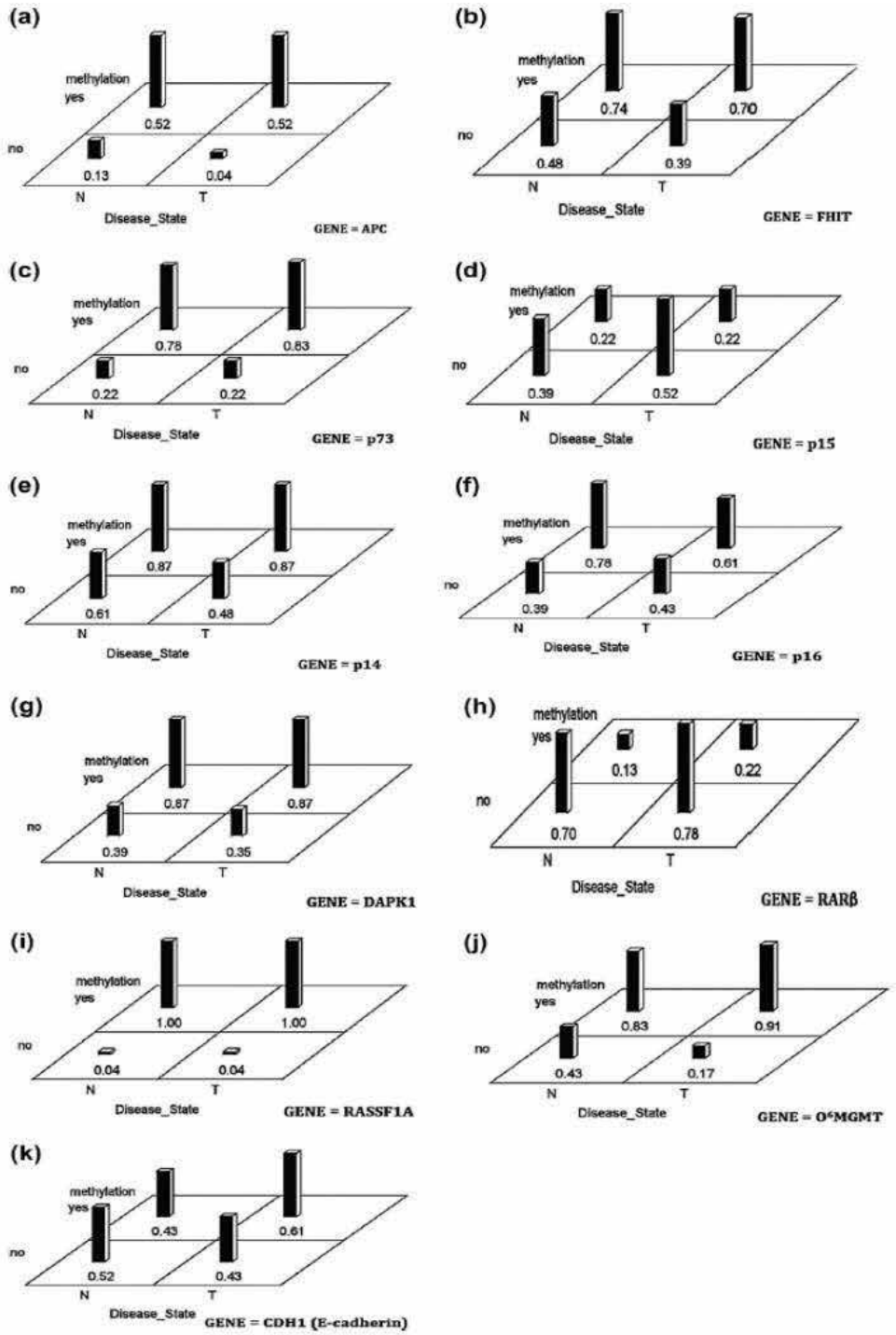

Figure 6. Differences across methylation profiles between HCC\cases and their ANT# samples with 0.0045 as a cut-off for significance. *HCC = T. # ANT = N. a-k: names of the studied genes.

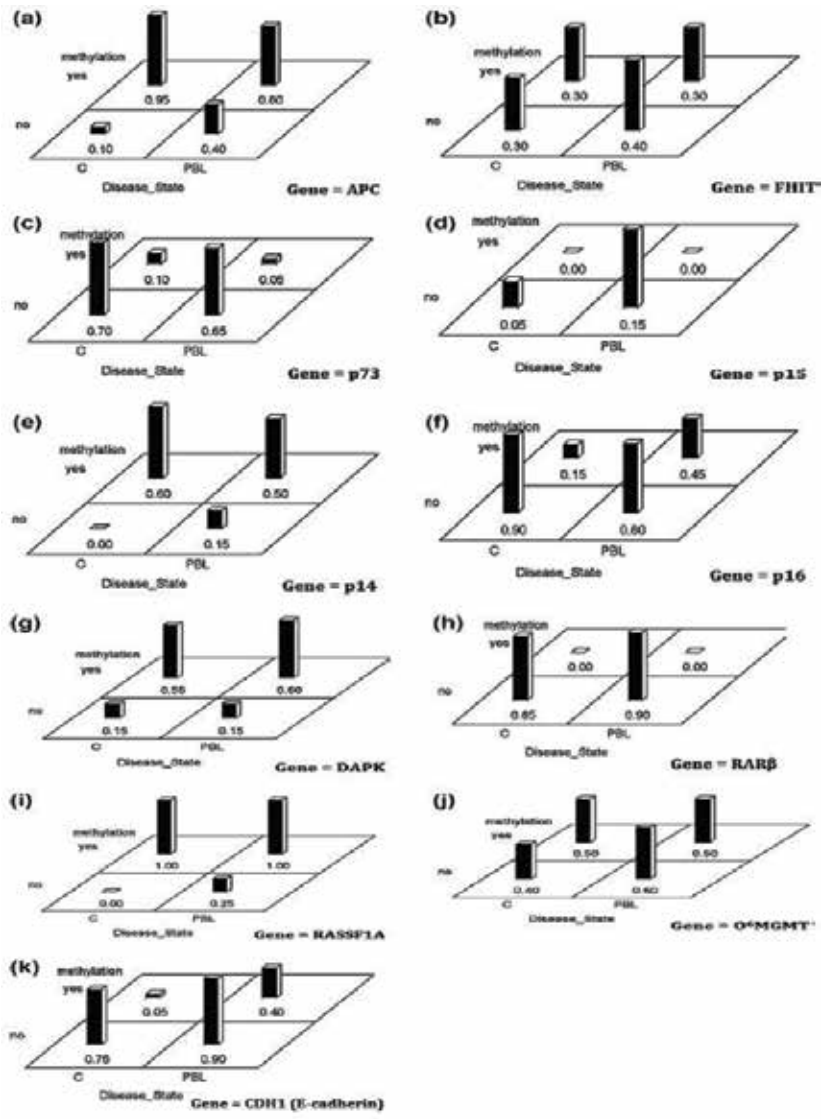

Figure 7. Differences across methylation profiles within CAH\cases between tissues and PBL. a-k: names of the studied genes.

6. Genes methylation could be used as a biomarker for diagnosis of HCC and CH

The Coordination of methylation at the 11 tested genes was analyzed in our study using the Mann–Whitney U test through comparing the status of each gene (M or U) with the MI calculated with the remaining genes (**Table 6**). The combined effect of the studied methylated genes as biomarkers for diagnosis of HCC and CH has been determined (assessed) using the stepwise logistic regression, and accordingly only ***APC, p73, p14 and O6MGMT*** independently affected the classification of cases into HCC and/or CH (**Table 7**). Together, these four genes (combined) give an accuracy of 89.9%, sensitivity 83.9% and specificity 94.7%.

Factor	Concordance, n=31 (n (%))	Kappa[#]	p-Value*
APC	28 (90.3)	0.803	<0.001
FHIT	24 (77.4)	0.497	0.006
P15	31 (100.0)	1.000	<0.001
P73	18 (58.1)	−0.248	0.150
P14	31 (100.0)	1.000	<0.001
P16	24 (77.4)	0.558	0.001
DAPK	22 (71.0)	0.318	0.076
RARb	27 (87.1)	0.431	0.012
RASSF	31 (100.0)	—	—
O6O6-MGMT	26 (83.9)	0.640	<0.001
CDH1	22 (71.0)	0.425	0.016

– Numbers are too small for a valid statistical analysis.[#]Kappa measure of agreement.
*p-Values<0.05 are considered significant.

Table 6. Summary of methylation specific PCR results and concordance tests of each locus in HCC samples.

Parameter	Regression estimate	P value	Odds ratio	95% CI	for OR
APC	−3.606	0.003	0.027	0.003	0.287
p73	3.671	0.001	39.302	4.752	325.017
P14	3.638	0.009	38.014	2.492	579.829
O6-MGMT	2.589	0.014	13.311	1.685	105.132

Table 7. Stepwise logistic regression for HCC.

Within the identified genes panel which independently affected the classification of cases into HCC and CH in this study, p14 only showed a high MF in HCC cases. Our data in this context confirmed those of Anzola et al. [30] and Yang et al. [20] who reported that ***p14*** PM is an important factor contributing for the development of HCV-induced HCC. The fact that we were able to detect ***p14***-PM in NHT and CH with almost the same frequency, suggests that it might be an early event in the cascade of HCV-induced HCC. On contrary, we could not find the same profile for ***p16***PM suggesting that ***p14*** and ***p16*** are may be regulated by different promoters [31].

Similar to ***p14, O6MGMT*** plays an important role in cytoprotection by preventing DNA damage and triggering DNA repair mechanisms [32]. Our results showed a significant increase in the frequency of ***O6MGMT*** PM from CH (26%) to HCC (67.7%) providing an evidence that this gene could be used to differentiate between CH and HCC. We have also reported that ***O6MGMT*** PM is significantly higher in non-responder to antiviral therapy, and consequently ***O6MGMT*** could be used as a predictor for antiviral response [33]. Literature reviews shows different frequencies of ***O6MGMT*** PM in HCC ranging from 0% to 22–39% [34, 35]. This variability in the results among different studies could be attributed to several factors including the sensitivity and type of PCR, the primer sequences used, the site of CpG islands, the geographical and the underlying etiological factors that promoting HCC development

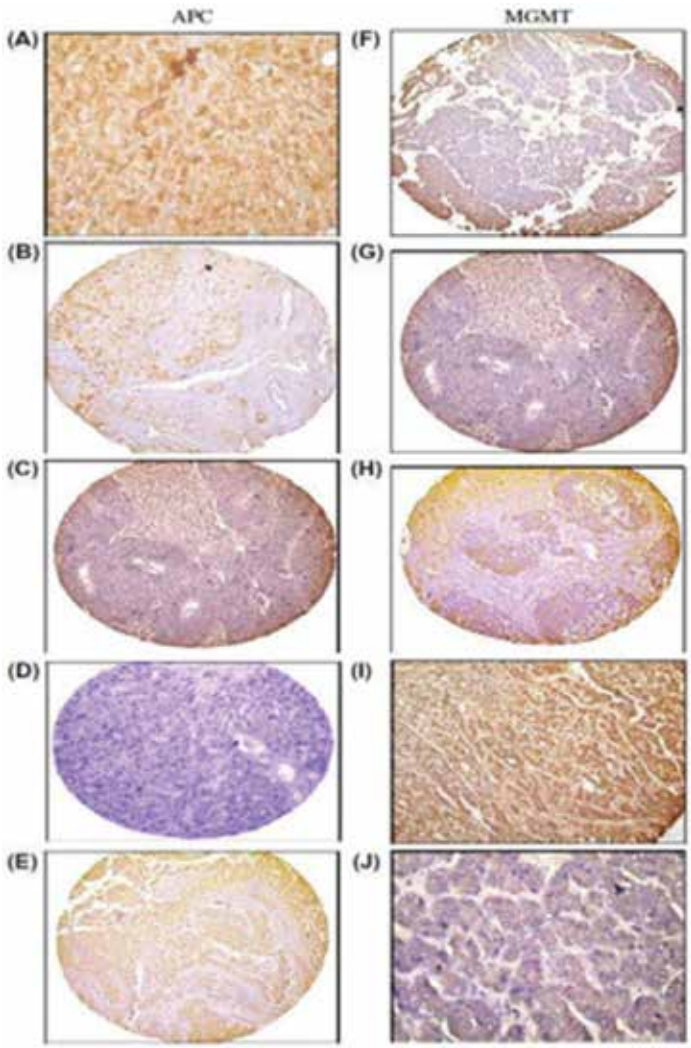

Figure 8. (A) Normal hepatic tissue sample showing positive cytoplasmic immunostaining for APC (200×). (B) A case of HCV induced chronic hepatitis showing mild focal cytoplasmic immunostaining for APC (100×). (C) A case of HCV induced chronic hepatitis with cirrhosis negative for APC (100×). D: A case of HCV-associated HCC negative for APC (100×). (E) A case of HCV-associated HCC with positive cytoplasmic immunostaining for APC (40×). (F) Normal hepatic tissue negative for MGMT (100×). (G) A case of HCV-induced chronic hepatitis with cirrhosis negative for MGMT (100×). (H) A case of HCV-induced chronic hepatitis with cirrhosis positive for MGMT immunostaining (100×). (I) A case of HCV-induced HCC with marked cytoplasmic immunostaining for MGMT (200×). (J) A case of HCV-induced HCC showing faint cytoplasmic immunostaining for MGMT (200×).

[24, 35]. And finally, ***p73*** PM was reported in 83.9% of the HCC cases assessed in our study compared to 21.1% in CH and none in the NHT samples. Thus ***p73*** PM could be used to differentiate between CH and HCC cases even in patient's blood [32].

A significant difference in the MFs of ***APC*** and ***CDH1*** were found between CH and HCC cases. APC was more frequent in the CH and ***CDH1*** in HCC. ***APC*** and ***CDH1*** PM was reported by Yang et al. [20] who demonstrated that PM of ***APC*** and ***CDH1*** are more frequent in HBV and

HCV-positive HCC than in HBV and HCV negative ones. Nomoto et al. [34] founded ***APC*** PM in 88.2% of the NHT and 21.6% in CH with cirrhosis compared to 82.4% in HCC. They explained that ***APC*** loss in cirrhotic and inflammatory cases could be occurred due to the presence of inflammatory cells and fibroblasts. However in contrast, we reported a high ***APC*** MF in the blood and tissues of CH patients. This contradictory between our results and those of Nomoto et al. could be attributed to (a) their smaller sample size (19 cases only); (b) the samples of CH and cirrhosis were obtained from HCC cases in their study or (c) a possibly different underlying etiology as viral infection was not mentioned in their study.

7. Concordance between PM and protein expression of APC and O6MGMT

We assessed the protein expression of ***APC*** and ***O6MGMT*** in 20 NHT samples, 20 HCC and 20 CH tissues as well as in another group of samples including 40 NHT, 52 CH and 107 HCC tissue samples for confirmation of the methylation results [21]. In the original set, cytoplasmic immunostaining for the ***APC*** was detected in 11 cases of NHT (55%), with loss of staining in 10 CH cases (50%), and 15 cases of HCC group (75%). As for the confirmatory set, cytoplasmic immunostaining for the APC was present in 50% of NHT (20 cases), with loss of staining in 57.7% of CH (30 cases), and 72% of HCCs (77 patients). Nuclear immunostaining for ***O6MGMT*** protein was detected in 13 patients with NHT (65%), with loss of expression in 11 patients with CH (55%) and 16 patients with HCCs (80%), from the original set. In the confirmatory set ***O6MGMT*** protein was lost in 26 patients with CH accounting for 50%, and 70 patients with HCC accounting for 65.4% (**Figure 8**).

8. Conclusion

We conclude that DNA PM of multiple cancer-related genes plays an important role in the development and progression of HCC and therefore, it could be detected in different stages of disease progression from hepatitis to HCC. The data reported in our study provide evidence that PM of *p73, p14, O6- MGMT* is associated with HCC whereas PM of the *APC* gene is more common in CH cases compared to other groups. Therefore, *APC* PM could be used as a maker for early detection of HCV-induced chronic active hepatitis patients.

Moreover, a panel of four genes *(APC, p73, p14, O6-MGMT)* independently affected the classification of cases into HCC and CH with high accuracy (89.9%), sensitivity (83.9%) and specificity (94.7%). In addition, detection of PM of certain genes *(APC, FHIT, p15, p16, and E-cadherin)* in the PBL of HCV-infected patients is a highly sensitive and specific, noninvasive way (technique) and therefore, blood could be used, as efficiently as tissue biopsies, to assess PM of different genes. This could help in the follow-up of chronic hepatitis patients and possibly for early detection of HCC. We did not observe a significant difference in the methylation status according to the virus type (HBV versus HCV infection). Therefore, plasma DNA could be used as a reliable resource for methylation studies in the future, irrespective of the type of hepatitis infection.

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References

[1] Siegel RL, Miller KD, Fedewa SA, Ahnen DJ, Meester RG, Barzi A, Jemal A. Colorectal cancer statistics, 2017. CA: A Cancer Journal for Clinicians. 2017 May 6;**67**(3):177-193. DOI: 10.1186/s13012-017-0668-7

[2] Ibrahim AS, Khaled HM, Mikhail NN, Baraka H, Kamel H. Cancer incidence in Egypt: Results of the national population-based cancer registry program. Journal of Cancer Epidemiology. 2014;**2014**

[3] Zekri AR, Hafez MM, Bahnassy AA, Hassan ZK, Mansour T, Kamal MM, Khaled HM. Genetic profile of Egyptian hepatocellular-carcinoma associated with hepatitis C virus genotype 4 by 15 K cDNA microarray: Preliminary study. BMC Research Notes. 2008 Oct 29;**1**(1):106. DOI: 10.1186/1756-0500-1-106

[4] Petruzziello A, Marigliano S, Loquercio G, Cozzolino A, Cacciapuoti C. Global epidemiology of hepatitis C virus infection: An up-date of the distribution and circulation of hepatitis C virus genotypes. World Journal of Gastroenterology. 2016;**22**:7824-7840. DOI: 10.3748/wjg.v22.i34.7824

[5] Chen SL, Morgan TR. The natural history of hepatitis C virus (HCV) infection. International Journal of Medical Sciences. 2006;**3**:47-52. DOI: 10.7150/ijms.3.47

[6] Zhang C, Li H, Zhou G, Zhang Q, Zhang T, Li J, et al. Transcriptional silencing of the TMS1/ASC tumour suppressor gene by an epigenetic mechanism in hepatocellular carcinoma cells. The Journal of Pathology. 2007;**212**(2):134-361. DOI: 10.1002/path.2173

[7] Ye M, Huang T, Ying Y, et al. Detection of 14-3-3 sigma (sigma) promoter methylation as a noninvasive biomarker using blood samples for breast cancer diagnosis. Oncotarget. 2017;**8**:9230-9242. DOI: 10.1002/path.2173

[8] Paska AV, Hudler P. Aberrant methylation patterns in cancer: A clinical view. Biochemical Medicine (Zagreb). 2015;**25**:161-176. DOI: 10.11613/BM.2015.017

[9] Li CC, Yu Z, Cui LH, et al. Role of P14 and MGMT gene methylation in hepatocellular carcinomas: A meta-analysis. Asian Pacific Journal of Cancer Prevention. 2014;**15**:6591-6596

[10] Teodoridis JM, Hardie C, Brown R. CpG island methylator phenotype (CIMP) in cancer: Causes and implications. Cancer Letters. 2008;**268**(2):177-355. DOI: 10.1016/j.canlet.2008.03.022

[11] Tsou JA, Galler JS, Wali A, Ye W, Siegmund KD, Groshen S, et al. DNA methylation profile of 28 potential marker loci in malignant mesothelioma. Lung Cancer. 2007;**58**(2): 220-230. DOI: 10.1016/j.lungcan.2007.06.015

[12] Lehman EM, Wilson ML. Epidemiology of hepatitis viruses among hepatocellular cases and healthy people in Egypt: A systematic review and meta-analysis. International Journal of Cancer. 2009;**24**:690-697. DOI: 10.1002/ijc.23937

[13] Iyer P, Zekri AR, Hung CW, Schiefelbein E, Ismail K, Hablas A, Seifeldin IA, Soliman AS. Concordance of DNA methylation pattern in plasma and tumor DNA of Egyptian hepatocellular carcinoma patients. Experimental and Molecular Pathology. 2010 Feb 28; **88**(1):107-111. DOI: 10.1016/j.yexmp.2009.09.012

[14] Bongiovanni M, Casana M. Non-invasive markers of liver fibrosis in HCV mono-infected and in HIV/HCV co-infected subjects. Medicinal Chemistry. 2008;**4**(6):513. [PubMed: 1899 1734]

[15] Huang W, Li T, Yang W, Chai X, Chen K, Wei L, Duan S, Li B, Qin Y. Analysis of DNA methylation in plasma for monitoring hepatocarcinogenesis. Genetic Testing and Molecular Biomarkers. 2015 Jun 1;**19**(6):295-302. DOI: 10.1089/gtmb.2014.0292

[16] Shen J, Wang S, Zhang YJ, Kappil M, Wu HC, Kibriya MG, Wang Q, Jasmine F, Ahsan H, Lee PH, Yu MW. Genome-wide DNA methylation profiles in hepatocellular carcinoma. Hepatology. 2012 Jun 1;**55**(6):1799-1808. DOI: 10.1002/hep.25569

[17] Liu JB, Zhang YX, Zhou SH, Shi MX, Cai J, Liu Y, Chen KP, Qiang FL. CpG island methylator phenotype in plasma is associated with hepatocellular carcinoma prognosis. World Journal of Gastroenterology: WJG. 2011 Nov 14;**17**(42):4718. DOI: 10.3748/wjg.v17.i42.4718

[18] Chang H, Yi B, Li L, Zhang H, Sun F, Dong S, et al. Methylation of tumor associated genes in tissue and plasma samples from liver disease patients. Experimental and Molecular Pathology. 2008;**85**(2):96. [PubMed: 18691570]

[19] Zekri AE, Nassar AA, El-Rouby MN, Shousha HI, Barakat AB, El-Desouky ED, Zayed NA, Ahmed OS, Youssef AS, Kaseb AO, El-Aziz AO. Disease progression from chronic hepatitis C to cirrhosis and hepatocellular carcinoma is associated with increasing DNA promoter methylation. Asian Pacific Journal of Cancer Prevention. 2013;**14**(11):6721-6726. PMID: 24377595

[20] Yang B, Guo M, Herman JG, Clark DP. Aberrant promoter methylation profiles of tumor suppressor genes in hepatocellular carcinoma. The American Journal of Pathology. 2003;**163**(3):1101-1107. DOI: 10.1016/S0002-9440(10)63469-4

[21] Zekri AR, Bahnasy AA, Mohamed WS, El-Dahshan DH, Ali FT, Sabry GM, Dasgupta N, Daoud SS. Methylation of multiple genes in hepatitis C virus associated hepatocellular carcinoma. Journal of Advanced Research. 2014 Jan 31;**5**(1):27-40. DOI: 10.1016/j.jare.2012.11.002

[22] Hesson LB, Cooper WN, Latif F. The role of RASSF1A methylation in cancer. Disease Markers. 2007;**23**:73-87. PMCID: PMC3850810

[23] Qu Z, Jiang Y, Li H, Yu DC, Ding YT. Detecting abnormal methylation of tumor suppressor genes GSTP1, P16, RIZ1, and RASSF1A in hepatocellular carcinoma and its clinical significance. Oncology Letters. 2015 Oct 1;**10**(4):2553-2558. DOI: 10.3892/ol.2015.3536

[24] Lia Z, Zhangb H, Yangb J, Haoc T, Li S. Promoter hypermethylation of DNA damage response genes in hepatocellular carcinoma. Cell Biology International. 2012;**36**:427-432. DOI: 10.1042/CBI20100851

[25] Araújo OC, Rosa AS, Fernandes A, Niel C, Villela-Nogueira CA, Pannain V, Araujo NM. RASSF1A and DOK1 promoter methylation levels in hepatocellular carcinoma, cirrhotic and non-cirrhotic liver, and correlation with liver cancer in Brazilian patients. PLoS One. 2016 Apr 14;**11**(4):e0153796. DOI: 10.1371/journal.pone.0153796

[26] Gioia S, Bianchi P, Destro A, Grizzi F, Malesci A, Laghi L, et al. Quantitative evaluation of RASSF1A methylation in the non-lesional, regenerative and neoplastic liver. BMC Cancer. 2006;**89**(6):1471-2407. DOI: 10.1186/1471-2407-6-89

[27] Chan KC, Lai PB, Mok TS, Chan HL, Ding C, Yeung SW, et al. Quantitative analysis of circulating methylated DNA as a biomarker for hepatocellular carcinoma. Clinical Chemistry. 2008;**54**:1528-1536. DOI: 10.1373/clinchem.2008.104653

[28] Lv X, Ye G, Zhang X, Huang T. p16 Methylation was associated with the development, age, hepatic viruses infection of hepatocellular carcinoma, and p16 expression had a poor survival: A systematic meta-analysis (PRISMA). Medicine. 2017 Sep;**96**(38):e8106. DOI: 10.1097/MD.0000000000008106

[29] Vivekanandan P, Torbenson M. Epigenetic instability is rare in fibrolamellar carcinomas but common in viral-associated hepatocellular carcinomas. Modern Pathology. 2008;**21**:670-675. DOI: 10.1038/modpathol.2008.32

[30] Anzola M, Cuevas N, López-Martinez M, Saiz A, Burgos JJ, Martinez de Pancorbo M. p14ARF gene alterations in human hepatocellular carcinoma. European Journal of Gastroenterology & Hepatology. 2004;**16**:19-26. PMID: 15095848

[31] Esteller M, Tortola S, Toyota M, Capella G, Peinado MA, Baylin SB, et al. Hypermethylation-associated inactivation of p14 (ARF) is independent of p16 (INK4a) methylation and p53 mutational status. Cancer Research. 2000;**60**:129-133. PMID: 10646864

[32] Lenz G, Hutter G, Hiddemann W, Dreyling M. Promoter methylation and expression of DNA repair genes hMLH1 and O6-MGMT in acute myeloid leukemia. Annals of Hematology. 2004;**83**:628-633. DOI: 10.1007/s00277-004-0925-0

[33] Zekri AR, Raafat AM, Elmasry S, Bahnassy AA, Saad Y, Dabaon HA, El-Kassas M, Shousha HI, Nassar AA, El-Dosouky MA, Hussein N. Promotor methylation: Does it affect response to therapy in chronic hepatitis C (G4) or fibrosis? Annals of Hepatology: Official Journal of the Mexican Association of Hepatology. 2014 Sep 1;**13**(5):518-524. PMID: 25152984

[34] Nomoto S, Kinoshita T, Kato K, Otani S, Kasuya H, Takeda S, et al. Hypermethylation of multiple genes as clonal markers in multicentric hepatocellular carcinoma. British Journal of Cancer. 2007;**97**:1260-1265. DOI: 10.1038/sj.bjc.6600743

[35] Matsukura S, Soejima H, Nakagawachi T, Yakushiji H, Ogawa A, Fukuhara M, Miyazaki K, Nakabeppu Y, Sekiguchi M, Mukai T. CpG methylation of MGMT and hMLH1 promoter in hepatocellular carcinoma associated with hepatitis viral infection. British Journal of Cancer. 2003 Feb 24;**88**(4):521-529. DOI: 10.1038/sj.bjc.6600743

Chapter 5

Micro-RNA in Hepatocellular Carcinoma - Related Hepatitis C Virus Patients in Correlation to Disease Progression

Moustafa Nouh Elemeery

Additional information is available at the end of the chapter

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Abstract

Hepatocellular carcinoma (HCC) is a multistep heterogeneous disease as it is related to the risk factors such as HBV and HCV infections, including uncontrolled hepatocyte proliferation, invasion of the neighboring tissue and metastasize to distant tissues. There are several factors affecting the course of HCC among the patients such as oncogenes and tumor suppressor genes. Recently, molecular mechanisms have cleared some of the underlying mechanisms of carcinogenesis, especially the microRNAs, the upstream regulators of a large number of critical genes. Mature miRNAs found to be mounted into RISC, which helps in recognizing the complementary binding sites in the 3′ untranslated regions of target genes. That binding causes the degradation of/or inhibition of translation of mRNAs. miRNAs have been reported to be deregulated in human cancers demonstrating their double-edged role as a tumor suppressor and as an oncogene. miRNA deregulation is involved in modulating signal pathways of cellular transformation of a normal cell into a cancer cell. miRNAs have been reported to be associated with the processes of carcinogenesis including inflammation, cell-cycle, differentiation, apoptosis, and metastasis. miRNAs have been considered as potential biomarkers in HCC as their development has been attributed to the deregulation of many genes owing to abnormal expression of miRNAs. Herein, the current chapter will focus on studying the regulation of miRNAs in HCC-related HCV patients.

Keywords: miRNA, HCC, HCV, UTR, fibrosis progression

1. Introduction

MicroRNA (miRNA) has been proven as key regulator homeostasis for multiple biological systems, besides modulation of the disease pathology of many cancers. Experimental target

miRNA biogenesis as key regulators using small molecules or other interferences sheds light on its crucial role in regulating posttranscriptional gene expression. Further studies reported the variability of their loci, the genetic organization, and their tissue specificity, besides controlling the translation of target protein and transcriptome in response to physiologic environmental cues, along with their vulnerability to become designated in diseases like cancer and fibrosis, including that related to infection viruses like HCV. Many pathways analysis of targeted genes performed using infection-associated miRNAs showed that the pathways related to signal transduction activation, DNA damage, and cell death were clearly observed in HBV-infected liver, while proteasome, lipid metabolism activation, immune response, and antigen presentation were predominantly in HCV-infected liver. These differences are associated with miRNAs' level in the infected liver and it was confirmed in cell line like Huh7.5 cells in which infectious HBV or HCV clones can be replicated, which proved that miRNAs act as key mediators of HCV and HBV infection and liver disease progression as well; therefore, miRNAs can act as liable therapeutic target molecules in the field of translational medicine.

2. Tissue-specific expression and variation level of miRNAs

MicroRNAs are a class of small, endogenous, conserved, non-coding RNAs with a length of 20–24 ribonucleotide RNA sequence that is biosynthesized through transcription of miRNA genes into primary transcripts (pri-miRNA), which are processed by the drosha, generating a precursor of a length of 70 nucleotides (pre-miRNA) with a hairpin-like structure. A remarkable mechanistic difference in canonical against noncanonical miRNAs is that canonical is drosha-dependent intronic miRNAs and so treated co-transcriptionally in the nucleus with protein-coding transcripts. Pre-miRNA is then processed by dicer in the cytoplasm generating mature miRNA duplexes. Mature miRNAs are then mounted into RISC (an miRNA-induced silencing complex) which helps in recognizing the complementary binding sites in the 3′UTR of target genes. Noncanonical intronic, ones called mirtrons, originate from small introns that are similar to pre-miRNAs and can detour the drosha-processing step [1, 2]. Noncanonical pathway affects common cellular response pathways like proliferation and apoptosis by targeting various mRNA transcripts [1]. miRNA binding causes the degradation of, or inhibition of, translation of mRNAs. miRNAs have been reported to be deregulated in human cancers demonstrating their double-edged role as a tumor suppressor and as an oncogene that offers miR clusters as complex and adaptive regulatory controllers for disease progression. Comparative research assessing the organizational structure for the mammalian genome has noticed enrichment in one of the following: copy number variation, chromosomal deletion or insertion, and single nucleotide polymorphisms (SNPs) that subsidize phenotypic diversity. This diversity is obvious in all aspects of human health and investigated diseases. No wonder there is a mounting gratitude to the variation in miRNAs and their target genes in phenotypic variability. Numerous solid malignancies that included hepatocellular carcinoma (HCC) proved to be correlated with miRNAs located at deleted,

amplified, or translocated chromosomal regions [3]. Variation in gene expression or regulation affected by expression of the quantitative trait loci is caused due to genetic variants in either cis- or trans-acting SNPs [4]. A remarkable criterion of miRNA binding is their capability to distinguish binding site polymorphism (miRSNPs) in transcribed functional genes, as in the case of miR 214-5p that appears to be dysregulated in HCC [2] and miR-24 in the case of colorectal tumor by the targeting site of polymorphism in the dihydrofolate reductase gene [5]. This binding causes inhibition of translation for its transcripts and can phenocopy the phenotypic character of such a disease with genetic knockouts of the responsible gene [5].

Screening miRNA genetic variation and differential expression level across the human population in healthy and disease patients provides more insights on variable causes of disease progression and susceptibility in addition to physical functionalities [4]. Comparative genomic studies showed that the untranslated regions (UTRs) within the mRNA sequence act as a target sequence even for mRNA-UTR-displaying variants; during miRNA-mRNA adaptive coevolution, the co-expressed miRNA selects its cognate UTR mRNA, which depends on whether the dysregulation of protein output will be harmful, beneficial or inconsequential for the desired effect [6].

Evaluating reports on tissue-specific differential expression of miRNAs showed the cross-regulation feature of miRNAs and its correlation to stability of phenotype differentiation [7], as an example, regulation of neurite outgrowth, dendritic spine size, and neural differentiation that is regulated by overexpression of miR219, miR134, miR128, miR24, miR7, and others [7]. In the same strategy, miR499, miR486, miR208, miR206, miR133, and miR1 proved to control skeletal muscle growth, maintenance and differentiation [8], while miR133 proved to inhibit osteogenic cell-linage differentiation through controlling Runx2 that is required for bone development, differentiation, and formation. Not only the previously mentioned roles but miRNAs can also exert specialized functions as in case of hypothalamus; fine-tuning expression of oxytocin; and Fos controlled by hyper-expression of both miR24 and miR7 and hence they control lactation and parturition through controlling water in the body [9].

MicroRNA (miRNA) has been proven as key regulator homeostasis for multiple biological systems, besides modulation of the disease pathology of many cancers. Experimental target miRNA biogenesis key regulators using small molecules or other interferences sheds light on its crucial role in regulating posttranscriptional gene expression. Further studies reported the variability of their loci, the genetic organization, and their tissue specificity [10], besides controlling the translation of target protein and transcriptome in response to physiologic environmental cues, along with their vulnerability to become designated in diseases like cancer and fibrosis, including that related to infection viruses like HCV. Many pathways analysis of targeted genes performed using infection-associated miRNAs showed that the pathways related to signal transduction activation, DNA damage, and cell death are clearly observed in HBV-infected liver, while proteasome, lipid metabolism activation, immune response, and antigen presentation were predominantly in HCV-infected liver [2, 3]. These differences are

associated with miRNAs' level in the infected liver and it was confirmed in cell line like Huh7.5 cells in which infectious HBV or HCV clones can be replicated, which proved that miRNAs act as key mediators of HCV and HBV infection and liver disease progression as well; therefore, miRNA can act as liable therapeutic target molecules in the field of translational medicine [11].

Since miRNA discovery as a liable promising class of small non-coding RNAs able to regulate protein translation and stability of mRNA, miRNAs have been implicated as key regulators in many diseases like cancer and autoimmune disease. So there is great effort to leverage knowledge of the miRNA regulatory system to these diseases, especially cancer [12].

3. MicroRNAs and disease susceptibility

Development in the pathobiology of miR Nas sheds light on its crucial character in transcriptome modulation which can reflect cancer state in addition to its application in attenuating possible risks that may be raised during cancer progression [13]. Currently, there is no doubt that down-regulating epithelial markers causes disruption in epithelial mesenchymal that is directly associated with differentiation of epithelial cells in lung cancer, a key developmental pathway in lung cancer progression and metastasis [14]. So expression of miRNA can be used as a progression marker for cancer disease depending on its differential expression during invasion, progression, and metastasis of cancer [3].

Reduction in dicer expression is often noticed in cancer stem cells like muscle stem cell tumors and rhabdomyosarcoma in periodic cases, shown to be correlated with the down-regulated level of myomiRNAs like miR 133 and miR 1 [15]. OncomiRNAs is a type of miRNA that can decrease tumor suppressor gene expression which leads to phenotype attenuation or promotion for oncogenic characters; miR92, miR21, and niR17 are members of that family that can modulate cell-cycle regulators like P21, PTEN, and E2F that can promote tumor proliferation [3, 12]. On the other hand, tumor-suppressor miRNAs, like let-7, target directly mRNA for silencing [3]; over-expressed Let7 has proved to modulate cell-cycle regulators that lead to tumor invasion and metastasis. So these miRNAs can be used as prognostic tools for cancer incidence or as a parameter for treatment susceptibility. In squamous cell carcinoma and adenocarcinoma, a comparative miRNA expression profiling revealed a significant difference that reached a specific signature to predict overall survival between male smoker patients in addition to the study performed by Liu et al. that clearly declared correlation between overall pathobiology in cancer and tissue-context miRNA expression [15]. Our team recently proved a small panel of four miRNAs that can act as a liable prognostic marker for HCC progression besides its ability to discriminate different stages in hepatocellular carcinoma [2].

3.1. miRNAs and hepatic diseases

Studies for understanding miRNAs in liver diseases showed a significant progression in that field, making liver a promising first organ to achieve precision and targeted therapy. Depending on accumulated studies of miRNA in liver disease, the unique vasculature of the

liver, and the efficiently rapid accumulation of exogenous small RNAs, the liver showed a good target for RNAi targeted therapy. Manipulation of miRNAs in liver diseases proved great evidences in that field; as an example miR122 clearly illustrates a good effective target for ameliorating hepatic steatosis besides many studies that showed miR122 to be a good target in HCV targeted therapy through its role in production of neutralizing antagomirs. MicroRNAs as a key target for viral hepatitis afford another liable possibility for targeting HBV and HCV infection that, by one way or another, causes HCC progression and death upon chronic infection; this targeting prevention will help to reduce HCC risk incidence by regulating many oncogenic miRNAs like miR222, miR221, and miR21 or via tumor suppression ones like miR199 and miR122 that nowadays are used as liable biomarkers in HCC, besides many promising studies that showed its role as prognostic and a marker for therapy response.

3.1.1. miRNA as a metabolic modulator in hepatic diseases

Non-alcoholic fatty liver diseases (NAFLD) are characterized by increased liver fat content and progression to liver inflammation, fibrosis, and ultimately cancer [16, 17]. Obesity, insulin resistance, and diabetes mellitus are risk factors for this disease and it is estimated that NAFLD will be the most common problem in internal medicine by 2020 [18]. Despite the high prevalence of NAFLD, the biology behind the disease progression is not clear and importantly there is no specific treatment for this condition, so osculating necessity for liable biomarkers and discovery of potential drug targets, as researched by Benhamouche-Trouillet et al., showed that miR-21 may be implicated at various steps during the NAFLD disease progression in a cell-specific manner through modulation of PPARα [19]. Specific conditional dicer1 deletion from embryonic liver leads to disruption in maturation of microRNA from its pre-microRNA form which leads to striking of metabolic phenotypes that include steatosis together with triglyceride and fatty acid accumulation in addition to dysregulation of blood glucose in fasting mice under study [12]. On the other hand, miR-355 has been recently considered as a liable biomarker for hepatic lipid accumulation in rat experiments as its elevated level strongly correlated with obesity in mice in association with liver steatosis [15]; for that reason, a high-throughput screen for miRNAs as a predictor for lipid droplet formation in the liver metabolic disorder in humans is a great demand for disease progression, overall survival rate, and even for prognosis of possible hepatic disorder (as shown in **Figure 1** and **Table 1**).

The conflicting concerns about whether profile expression of miRNA correlated with NASH or NAFLD have also been well investigated. Sanyal [20] research group reported in double subject groups, including NASH and metabolic syndrome group, in addition to the control group; control groups were matched in BMI. His investigators reported 23 up-regulated miRNAs and the same number of down-regulated miRNAs, with detailed interpretation referring to the role of some miRNA expression dysregulation, that is, miR-34a and miR-146b up-regulation besides miR-122 down-regulation in NASH subjects. Odd findings were reported in human subjects with NASH-like decreased expression of miR-122. However, the protective feature arise from silencing of miR-122 specially in high fat-fed mice bears only indirect physiological matter differentiate steady-state cross-sectional investigations in overweight or obese humans subjects with such a fatty liver disease. This result includes the complexity of dissecting effects and causes cross-talk in hepatic expression of miR-122 and metabolic liver disease.

The importance of miRNAs cross-talk analysis was further elucidated in many publications where hepatic steatosis variations was evolved through application of an adenovirus encoding a dominant negative c-Jun and then testing changes in miRNA expression that is associated with it [21]. They found many miRNAs (miR-122 and miR-370 was common among many publications) to be differentially expressed (DE) in liver tissues of mice that were treated by adenovirus and showed that the elevated presence of miR-370 correlated with the osculating expression of hepatic lipogenic target mRNAs (e.g., FAS, SREBP-1c, and DGAT2); these findings suggest that dietary modulation of some miRNA expression is a relevant consideration [21].

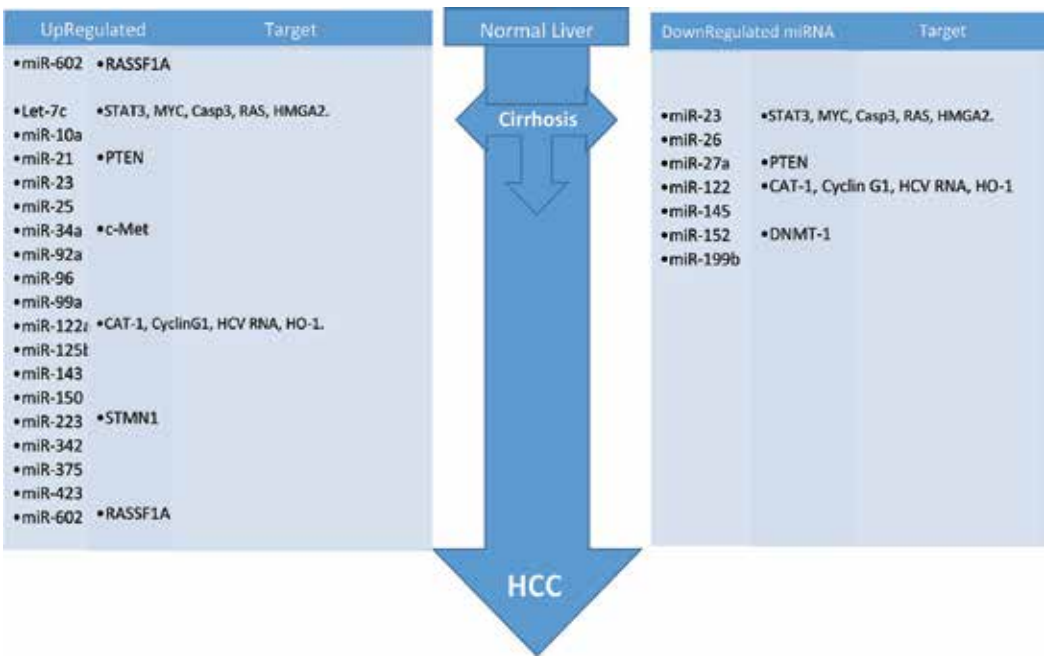

Figure 1. miRNA that either up-regulated or down-regulated with its target genes in different stages of HCC disease progression.

Liver diseases	**Down-regulated miRNAs**	**Up-regulated miRNAs**	**Dysregulated miRNA**
Steatohepatitis	miR-21	miR-17	miR-21
	miR-29a	miR-24	miR-33a/b
	miR-130a	miR-27	miR-122
	miR-185	miR-34a	miR-155
	miR-205	miR-103	
	miR-206	miR-107	
	miR-378	miR-122	
	miR-451		

Liver diseases	**Down-regulated miRNAs**	**Up-regulated miRNAs**	**Dysregulated miRNA**
HCV infection	Let-7	miR-21	miR-126
	miR-17	miR-122	miR-192
	miR-27a	miR-141	miR-198
	miR-29a,b,c	miR-146a	miR-345
	miR-130a	miR-192	
	miR-155	miR-215	
	miR-181a	miR-491	
	miR-194		
	miR-196		
	miR-199a		
	miR-221		
HCC	Let-7	miR-18a	miR-233
	miR-1	miR-21	
	miR-15a	miR-92a	
	miR-16	miR-130b	
	miR-26a	miR-141	
	miR-29	miR-155	
	miR-34a	miR-181	
	miR-101	miR-195	
	miR-122	miR-221	
	miR-124	miR-222	
	miR-125b	miR-224	
	miR-126	miR-494	
	miR-138	miR-1269	
	miR-141		
	miR-145		
	miR-146a		
	miR-148		
	miR-195		
	miR-199		
	miR-200		
	miR-223		
	miR-375		

Table 1. Liver diseases in association with the microRNA level in each stage.

3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), the enzyme that catalyzes mevalonic acid synthesis rate-limiting step in cholesterol and other isoprenoid production, showed both miR-21 and miR-34a as key player molecules in this step, through controlling dephosphorylation and activation of HMGCR [22]. One requirement for any effort to enhance miRNA levels as therapeutic tools is the well-established pro-oncogeneic characters for miRNAa in HCC and its related diseases, which will be discussed later here.

3.1.2. miRNA and HCV infection susceptibility

Hepatitis C virus is the sole member of hepacivirus C species that is known to be a blood-borne infectious viral disease causing the significant persistence of liver disease, with around 110–170 million infected patients globally, with nearly two-thirds of this number chronically infected and not less than one-third developing fibrosis and cirrhosis after 20 years of the onset of infection; most of them develop different stages of hepatocellular carcinoma [23]. Possible therapy for chronic hepatitis C (CHC) virus treatment has undergone a great transformation recently; discoveries in viral infections in humans have shown surprising findings that have broadened our understanding of miRNA function within human body.

In the setting of the HCV infection, the role of various miRNAs in modulating the viral infection response has been deeply studied, that clarifies causes of chronic hepatitis C progression in most infected patients and consequences of infection with its manipulation in the risk of developing cirrhosis and HCC [24]. The HCV virus is a positive-sense, single-stranded RNA virus of 9600 base [25]. It contains 5′ untranslated region (UTR) that contains four structurally conserved domains besides an internal ribosomal entry site (IRES) which allows viral RNA translation in a cap-independent manner with minimal dependence on canonical translation factors [26]. Translation of viral RNA leads to a polyprotein product that consists of six nonstructural and four structural viral proteins that undergo additional proteolysis by viral and host enzymes [27].

Subgenomic systems are easier to proceed after discovery of first sustainable cell-culture models for hepatitis viruses, in 1999 [28]. A noticed curious aspect in these early sustainable replicon systems was the successful sustainability of viral replication in Huh7 cell line but not HepG2, albeit both of these transformed cell lines have their origin in hepatocellular cancer in humans. The biologic declaration for this conflicted efficiency was first explained by Jopling et al. in 2005. When he verified that miR-122 has a detectable level in Huh7 but not HepG2 [29], in addition to that, he recently noticed that HCV contains a recognition site for the miR-122's seed sequence in the UTR area of viral genome. The miR-122-interacted viral elements have been mapped to two conserved points within 5′ UTR among stem-loop I and/or II, corresponding to the seed sequence of the miR-122 [30]. The finding was more astounding on the grounds that it appeared to be illogical to the customary thought of RNAi as an innate antiviral response like in invertebrates or plants [31]. Inhibition of RISC effector complex molecules like drosha, dicer1, DGCR8, and the RISC using small interfering RNA (siRNA) appears to inhibit HCV replication [32]. Although the mechanism underlying the miR-122 interaction with HCV is not precisely understood, miR-122 binding site position within the 5′ UTR proved to be critical, so translocation of this site to the 3′ UTR in a luciferase reporter mRNA causes up-regulation in reporter activity upon miR-122 diminished levels [30]. MiR-122 has

been assumed to elevate both replication and translation of RNA, independently from viral replication [33]. Up-regulation of the translation step through miR122 dependent pathway is observed in reporter and full-length HCV genome constructs [34]. Also, Jangra et al. [35] deliberated the mutations of full-length HCV constructs that are capable of generating infectious virions in vitro. He found zero overlapped mutations within the miRNA or IRES binding site in distinct constructs. In those harboring mutations in IRES, infective viral production was down-regulated by more than 28-fold in comparison with constructs with the miR-122 binding site disruption that showed more than a 3000-fold reduction [35]. These observations are important in description of the role of miR-122 in HCV infection that requires searching beyond HCV replication, translation, and stability to investigate more pathways like liable RNA targets in HCV biology or posttranslational targets for it, as an example, heme oxygenase-1 (HO-1) which catalyzes the degradation of heme to biliverdin. Oxidative stress causes HO-1 elevation. Incubation of HCV-infected cell lines with biliverdin causes reduction in HCV amplicons via stimulation of interferon pathways [36]. Heterodimers of BACH1 and a member of the Maf protein family cause transcription repression of HO-1. It was observed that BACH1 3′ UTR contains miR-122 binding sites; its function was confirmed to be important by silencing miR-122 that leads to increased HO-1 mRNA levels double fold. Not only that but BATCH1 silencing using siRNA or other chemical means like heme or cobalt protoporphyrin also decreased HCV RNA level [37].

However, in these research-based findings, miR-122 proved to be essential in replication of HCV. Later cloned HCV from other genotypes proved to be replicated in some cell lines like HepG2 cells [38], liver cells (hepa1–6) [39], and cervical cancer-derived HeLa cell from humans [40]. In addition, the findings from cell culture are not yet completely correlated with outcomes from clinical infection, like miR-122 level in liver tissue of infected patients and viral load [41]. Moreover, HCV therapy non-responder (NR) patients showed lower pretreatment miR-122 levels in liver tissue biopsies rather than responders [41]. Another study showed inverse correlation between severity of hepatic fibrosis and hepatic miR-122 expression levels [42]. Even bearing in mind those conclusions, there is still convincing evidence that miR-122 targeted therapy may act as a liable strategy in HCV precision medicine. For example, Lanford et al. treated chronically infected chimpanzees with an LNA-modified oligonucleotide against miR-122 [43]. There was a significant drop by 2.6 orders of magnitude of HCV in the chimpanzee that received the optimal dose of this agent besides significant improvement in histologic examination of the liver specimens. Additionally, 5′ UTR sequencing indicated no signs for selection of adaptive mutations to the recognition site of miR-122. Similar findings were also reported in human clinical trials, phase II, that test the LNA-modified phosphorothioate antisense DNA oligonucleotide and the anti-miR-122 antagomir (Miravirsen) [44]. These findings were so encouraging to researchers, where the Miravirsen subcutaneous injection reduces serum HCV viral load in a dose-dependent manner, up to a 3-log reduction over 2–5 months [44]. Small molecules synthesis that targets miR-122 raises the possibility for new opportunities in HCV infection treatment [45].

However, miR-122 is the finest studied HCV-related miRNAs; it is not sole. There are others like MiR-199a that recognize HCV 5′ UTR and so suppress viral load [46]. Also, MiR196 down-regulates BACH1 [47], and MiR-196 is up-regulated in response to interferon

signaling [48]. Besides, immune-regulatory miRNA-155 is induced by antiviral TLR3/4 signals [49]. Elevated MiR-155 levels in HCV-infected patients appeared to be inversely proportional to serum viral loads, signifying its relevant antiviral effect through suppression of Tim-3 (HAVCR2) that acts as an immune signaling modulator that is elevated in NK cells of HCV-infected patients where its up-regulation leads to inhibition of Tim-3 that causes an escalating production of interferon-γ (IFNγ) [50]. Not only that but MiR-155 also shows antiviral effects against other viruses like HIV infection through TLR3/TLR4 signaling pathway that prevents macrophage infection by HIV. Last but not least, miR-21, like many miRNAs, is induced by HCV infection but its induction aids HCV and escapes the immune response through directly suppressing interleukin-1 receptor-associated kinase 1 (IRAK1) and myeloid differentiation factor 88 (MyD88) [51] that is mandatory for mediating induced interferon response (type I) upon HCV infection. So, augmentation of miRNA expression levels and activity may prove a valuable aspect in HCV treatment and probably other viral infections.

4. MicroRNA inhibitors as a promising therapeutic approach

From the therapeutic point of view, silencing some miRNAs that encode potential vital or good protein-coding genes that is mandatory to preserve our health status. Inhibitors for these miRNAs have been considered for prospective therapeutic agents. Several approaches include direct delivery of miRNA-ASO or expressing it via mini-circle or viral vectors, which have been recognized for effective miRNA knockdown in vitro and in vivo too. Lately, miR-ASO-based therapy has been applied in humans and promising ongoing clinical trials considering liver sicknesses [52].

5. Conclusions

In conclusion, miRNA is a vital feature of assurance and monitoring throughout the tissue growth and disease states. In the near term, there will be much to be learned about adaptive or maladaptive states by an investigative way of differential expression of many miRNAs that is affected by the miRNA genetic architecture, mirtrons, and clusters in addition to SNP in miRNA or polymorphism in their target mRNA. There are diverse approaches of miRNA regulatory mechanism of action, for example negative, positive feedback and cross-regulatory through which various biological processes can be monitored, modulated or even resolved its signaling pathways, include fibrosis, viral infection and cancer. Possibly the micromanagement and homeostasis of these systems of regulatory miRNAs, when disturbed, can attain novel wannabe steady state of interacted interfaces that show an undesired effect in disease progression and severity especially in viral-related cancer cases like HCC. So, an enhanced sympathetic of these miRNA regulatory networks, in addition to improved therapeutic tools for controlling miRNA expression or their targets toward healthy regulatory states, will gain

more interest over the coming years. Indeed, modern advanced miRNA precision-based medicine will undergo advanced phases of clinical trials that will afford more understandings into the biosafety and bioavailability in addition to the efficacy of miRNA as therapy and diagnostic tools.

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Conflict of interest

The author declares that there is no conflict of interest for this chapter.

Acronyms and abbreviations

CHC	chronic hepatitis C
E2F	elongation factor 2
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HO-1	heme oxygenase-1
HMG-CoA	3-hydroxy-3-methyl-glutaryl-CoA
IRES	internal ribosomal entry site
LNA	locked nucleic acid
miR	micro-ribonucleic acid
NAFLD	non-alcoholic fatty liver diseases
NASH	non-alcoholic steatohepatitis
PTEN	phosphatase and tensin homolog
RISC	RNA-induced silencing complex
UTR	untranslated region

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References

[1] Dai R, Ahmed SA. MicroRNA, a new paradigm for understanding immunoregulation, inflammation, and autoimmune diseases. Translational Research. 2011;**157**(4):163-179

[2] Elemeery MN et al. Validation of a serum microRNA panel as biomarkers for early diagnosis of hepatocellular carcinoma post-hepatitis C infection in Egyptian patients. World Journal of Gastroenterology. 2017;**23**(21):3864

[3] Nana-Sinkam SP, Croce CM. MicroRNAs as therapeutic targets in cancer. Translational Research. 2011;**157**(4):216-225

[4] Zhou T, Garcia JG, Zhang W. Integrating microRNAs into a system biology approach to acute lung injury. Translational Research. 2011;**157**(4):180-190

[5] Clop A et al. A mutation creating a potential illegitimate microRNA target site in the myostatin gene affects muscularity in sheep. Nature Genetics. 2006;**38**(7):813

[6] Bartel DP. MicroRNAs: Target recognition and regulatory functions. Cell. 2009;**136**(2): 215-233

[7] Lian JB et al. MicroRNA control of bone formation and homeostasis. Nature Reviews Endocrinology. 2012;**8**(4):212

[8] Townley-Tilson WD, Callis TE, Wang D. MicroRNAs 1, 133, and 206: Critical factors of skeletal and cardiac muscle development, function, and disease. The International Journal of Biochemistry & Cell Biology. 2010;**42**(8):1252-1255

[9] Meister B, Herzer S, Silahtaroglu A. MicroRNAs in the hypothalamus. Neuroendocrinology. 2013;**98**(4):243-253

[10] Ryan DG, Oliveira-Fernandes M, Lavker RM. MicroRNAs of the mammalian eye display distinct and overlapping tissue specificity. Molecular Vision. 2006;**12**(134):I175-I184

[11] Ura S et al. Differential microRNA expression between hepatitis B and hepatitis C leading disease progression to hepatocellular carcinoma. Hepatology. 2009;**49**(4):1098-1112

[12] Laurence J. Translating MicroRNAs to the Clinic. London, United Kingdom: Academic Press; 2016

[13] Aoi W, Sakuma K. Does regulation of skeletal muscle function involve circulating microRNAs? Frontiers in Physiology. 2014;**5**:39

[14] Cortez MA et al. MicroRNAs in body fluids—The mix of hormones and biomarkers. Nature Reviews. Clinical Oncology. 2011;**8**(8):467

[15] Liu X et al. Involvement of microRNAs in lung cancer biology and therapy. Translational Research. 2011;**157**(4):200-208

[16] Kleiner DE et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005;**41**(6):1313-1321

[17] Day CP. From fat to inflammation. Gastroenterology. 2006;**130**(1):207-210

[18] Guerrero R et al. Ethnic differences in hepatic steatosis: An insulin resistance paradox? Hepatology. 2009;**49**(3):791-801

[19] Benhamouche-Trouillet S, Postic C. Emerging role of miR-21 in non-alcoholic fatty liver disease. Gut. 2016;**65**:1781-1783. gutjnl-2015-310044

[20] Sanyal AJ et al. Nonalcoholic steatohepatitis: Association of insulin resistance and mitochondrial abnormalities. Gastroenterology. 2001;**120**(5):1183-1192

[21] Iliopoulos D et al. MicroRNA-370 controls the expression of microRNA-122 and Cpt1α and affects lipid metabolism. Journal of Lipid Research. 2010;**51**(6):1513-1523

[22] Min H-K et al. Increased hepatic synthesis and dysregulation of cholesterol metabolism is associated with the severity of nonalcoholic fatty liver disease. Cell Metabolism. 2012;**15**(5):665-674

[23] Thrift AP, El-Serag HB, Kanwal F. Global epidemiology and burden of HCV infection and HCV-related disease. Nature Reviews. Gastroenterology & Hepatology. 2017;**14**(2):122

[24] Cazanave SC et al. A role for miR-296 in the regulation of lipoapoptosis by targeting PUMA. Journal of Lipid Research. 2011;**52**(8):1517-1525

[25] Miller AM et al. MiR-155 has a protective role in the development of non-alcoholic hepatosteatosis in mice. PLoS One. 2013;**8**(8):e72324

[26] Sharma H et al. Expression of genes for microRNA-processing enzymes is altered in advanced non-alcoholic fatty liver disease. Journal of Gastroenterology and Hepatology. 2013;**28**(8):1410-1415

[27] Alter MJ et al. Risk factors for acute non-A, non-B hepatitis in the United States and association with hepatitis C virus infection. Journal of the American Medical Association. 1990;**264**(17):2231-2235

[28] Wree A et al. Obesity affects the liver—The link between adipocytes and hepatocytes. Digestion. 2011;**83**(1-2):124-133

[29] Yamada H et al. Associations between circulating microRNAs (miR-21, miR-34a, miR-122 and miR-451) and non-alcoholic fatty liver. Clinica Chimica Acta. 2013;**424**:99-103

[30] Tryndyak VP et al. Plasma microRNAs are sensitive indicators of inter-strain differences in the severity of liver injury induced in mice by a choline-and folate-deficient diet. Toxicology and Applied Pharmacology. 2012;**262**(1):52-59

[31] Gerhard GS, DiStefano JK. Micro RNAs in the development of non-alcoholic fatty liver disease. World Journal of Hepatology. 2015;**7**(2):226

[32] Ceccarelli S et al. Dual role of microRNAs in NAFLD. International Journal of Molecular Sciences. 2013;**14**(4):8437-8455

[33] Volinia S et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proceedings of the National Academy of Sciences of the United States of America. 2006;**103**(7):2257-2261

[34] Tomimaru Y et al. MicroRNA-21 induces resistance to the anti-tumour effect of interferon-α/5-fluorouracil in hepatocellular carcinoma cells. British Journal of Cancer. 2010;**103**(10): 1617

[35] Karakatsanis A et al. Expression of microRNAs, miR-21, miR-31, miR-122, miR-145, miR-146a, miR-200c, miR-221, miR-222, and miR-223 in patients with hepatocellular carcinoma or intrahepatic cholangiocarcinoma and its prognostic significance. Molecular Carcinogenesis. 2013;**52**(4):297-303

[36] Gramantieri L et al. MicroRNA involvement in hepatocellular carcinoma. Journal of Cellular and Molecular Medicine. 2008;**12**(6a):2189-2204

[37] Hu S et al. MicroRNA-21 promotes cell proliferation in human hepatocellular carcinoma partly by targeting HEPN1. Tumor Biology. 2015;**36**(7):5467-5472

[38] Bao L et al. MicroRNA-21 suppresses PTEN and hSulf-1 expression and promotes hepatocellular carcinoma progression through AKT/ERK pathways. Cancer Letters. 2013;**337** (2):226-236

[39] Zhang Z et al. microRNA-146a inhibits cancer metastasis by downregulating VEGF through dual pathways in hepatocellular carcinoma. Molecular Cancer. 2015;**14**(1):5

[40] Rong M et al. Expression and clinicopathological significance of miR-146a in hepatocellular carcinoma tissues. Upsala Journal of Medical Sciences. 2014;**119**(1):19-24

[41] Murakami Y et al. Comprehensive analysis of microRNA expression patterns in hepatocellular carcinoma and non-tumorous tissues. Oncogene. 2006;**25**(17):2537

[42] Pineau P et al. miR-221 overexpression contributes to liver tumorigenesis. Proceedings of the National Academy of Sciences. 2010;**107**(1):264-269

[43] Fornari F et al. MiR-221 controls CDKN1C/p57 and CDKN1B/p27 expression in human hepatocellular carcinoma. Oncogene. 2008;**27**(43):5651

[44] Gramantieri L et al. MicroRNA-221 targets Bmf in hepatocellular carcinoma and correlates with tumor multifocality. Clinical Cancer Research. 2009;**15**(16):5073-5081

[45] Bae HJ et al. MicroRNA-221 governs tumor suppressor HDAC6 to potentiate malignant progression of liver cancer. Journal of Hepatology. 2015;**63**(2):408-419

[46] Morishita A, Masaki T. miRNA in hepatocellular carcinoma. Hepatology Research. 2015;**45**(2):128-141

[47] Jopling C. Liver-specific microRNA-122: Biogenesis and function. RNA Biology. 2012; **9**(2):137-142

[48] Wang B et al. Reciprocal regulation of microRNA-122 and c-Myc in hepatocellular cancer: Role of E2F1 and transcription factor dimerization partner 2. Hepatology. 2014; **59**(2):555-566

[49] Coulouarn C et al. Loss of miR-122 expression in liver cancer correlates with suppression of the hepatic phenotype and gain of metastatic properties. Oncogene. 2009;**28**(40):3526

[50] Zoni E et al. Epithelial plasticity in cancer: Unmasking a MicroRNA network for TGF-β-, Notch-, and Wnt-mediated EMT. Journal of Oncology. 2015;**2015**

[51] Liu S et al. MicroRNA-135a contributes to the development of portal vein tumor thrombus by promoting metastasis in hepatocellular carcinoma. Journal of Hepatology. 2012; **56**(2):389-396

[52] Kota SK, Balasubramanian S. Cancer therapy via modulation of micro RNA levels: A promising future. Drug Discovery Today. 2010;**15**(17-18):733-740

Section 3

Hepatitis C and Associated Clinical Implications

Chapter 6

Metabolic Factors and Their Influence on the Clinical Course and Response to HCV Treatment

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Additional information is available at the end of the chapter

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Abstract

Nowadays, direct-acting antivirals (DAA) have been used for hepatitis C virus (HCV) treatment leading to cure in 90–95% of non-cirrhotic patients depending on genotype, treatment experience, and regimen used. It was observed rates of antiviral response above 90% in compensated cirrhotic patients that should be treated for long time and/or ribavirin may be required. Metabolic syndrome, obesity, and insulin resistance are increasing worldwide and further contribute to hepatic steatosis and have long been recognized as a cause of lipid deposition in the liver. These factors affect the rate of antiviral response to interferon-based therapy, but it seems not impact DAA treatment. The effect of HCV eradication on hepatic steatosis and progression to fibrosis, cirrhosis, and hepatocellular carcinoma warrants further study in the era of direct-acting antivirals. Other factors that could be related to increase liver damage are vitamin D and associated polymorphisms. Patients with low concentration of total vitamin D [25(OH)D] presented high degree of fibrosis and high values of total cholesterol and triglycerides. In this chapter, we review the challenges and metabolic pathology associated with HCV infection and, discuss the influence of some metabolic factors which can cause liver damage.

Keywords: hepatitis C, metabolic syndrome, insulin resistance, vitamin D, genetic polymorphism

1. Introduction

Hepatitis C virus (HCV) infection is a serious health problem with an estimated 71 million of people having chronic HCV worldwide [1]. During chronic hepatitis C (CHC), it is observed many extrahepatic manifestations that could lead to rapid progression of the

disease, increasing the risk of developing hepatocellular carcinoma (HCC) and advanced fibrosis [2, 3]. The effect of HCV eradication on hepatic steatosis and progression to fibrosis, cirrhosis, and hepatocellular carcinoma warrants further study in the era of direct-acting antivirals. Now, with HCV eradication possible in virtually everyone, the sequelae of steatosis, fibrosis and its drivers will garner more attention. People infected by HCV genotype other than three presenting high BMI and visceral obesity have high risk of hepatic steatosis. It is believed that insulin resistance (IR) is the primary pathologic mechanism that leads to abnormal lipid accumulation within hepatocytes. But it is not defined if IR is due to host factors, presence of HCV infection, or a combination. These data become extremely relevant due to the high prevalence of obesity and metabolic syndromes observed worldwide [3].

Nowadays antiviral treatment for HCV demonstrated to be very effective (>90%), but it is important to recognize and identify irreversible and associated metabolic damage, thereby reducing the morbidity and mortality associated with HCV [3]. IR has been associated to CHC [4, 5], which is characterized by hyperinsulinemia in patients with normal fasting blood glucose and with an increased risk of developing diabetes mellitus type II (DM2), heart disease, and nonalcoholic fatty liver disease [6–8].

One of the consequences of persistent IR may be the development of DM2. DM2 is a metabolic disease characterized by hyperglycemia that can occur due to defects in insulin secretion and/or action involving specific pathogenic processes, such as the destruction of insulin-producing pancreatic beta cells or resistance to insulin action. It is the most common metabolic disease and the one with the highest prevalence among individuals with hepatitis C compared to those infected with the hepatitis B virus (HBV), for example [9, 10]. DM2 comprises approximately 90% of cases and may have a genetic and environmental component. Type 1 diabetes, comprising about 10% of the cases, results in the destruction of beta cells, which may lead to absolute insulin deficiency, thus requiring the exogenous administration of it to avoid ketoacidosis and coma.

HCV core protein is involved in the development of IR, however little is known about the clinical impact of HCV core region on IR [11, 12]. Patients infected with HCV genotype 1b who had 70Q core mutation had higher rates of IR compared to those without the mutation, indicating that this substitution is associated with the development of IR [11]. Mutation at core 70Q have been associated to higher incidence of HCC and mutations in 70 and/or 91 core HCV are important predictors of IR in patients without cirrhosis or DM [13, 14]. However, this finding was not seen in Brazilian population [12].

Other factor that could be related to increase liver damage is vitamin D and associated polymorphisms. Vitamin D, whose active form is 1,25-dihydroxy vitamin D3, is essential for calcium and bone homeostasis, and its deficiency has been associated to several diseases, such as cancer, cardiovascular and autoimmune diseases, IR, and infectious disease [15–19].

Vitamin D is an important immunomodulator and plays an important role in metabolic and inflammatory diseases in the liver, including HCV infection. Vitamin D deficiency is common in healthy worldwide populations [20]. Despite this, patients with liver diseases such as CHC are at substantially higher risk for hypovitaminosis D [15, 21, 22]. The polymorphism of the

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vitamin D receptor (VDR) gene was associated with rapid progression to fibrosis [bAt haplotype (CCA)] among HCV patients [23]. This information together demonstrates the potential of VDR-vitamin D axis association in viral hepatitis and highlights the importance of vitamin D as an immunomodulator, indicating an association between vitamin D deficiency and the absence of sustained virological response (SVR) in patients with hepatitis C [24, 25].

Studies have found a relationship between vitamin D concentration and decreased response to antiviral treatment in hepatitis C patients with genotype 1, 2 and 3 in double therapy with peg-interferon (PEG-IFN) and ribavirin [15, 24]. Bitetto et al. [26] observed that the vitamin D concentration and polymorphism in rs12979860 of IL28B gene were independent predictors of response to treatment. Patients who did not present the CC (IL28B) and vitamin D deficient genotype presented a greater risk of not responding to antiviral treatment. In addition, vitamin D concentration supplementation improves response to antiviral treatment in double therapy with PEG-IFN and ribavirin for recurrent hepatitis C [27]. Scalioni et al. [28] demonstrated that patients with lower concentration of 25(OH)D presented high degree of fibrosis and higher values of total cholesterol and triglycerides.

Currently, studies have been conducted correlating vitamin D and SVR levels in patients under direct-acting antivirals (DAAs) treatment. Backsteadt et al. [29] evaluated the association of vitamin D levels with cirrhosis in an HCV-infected cohort. In addition, they assessed pre-treatment vitamin D levels up to week 12. A higher prevalence of vitamin D deficiency was observed in cohorts of HCV-cirrhotic patients, but changes in vitamin D levels did not influence SVR rates [29]. Belle et al. [30] evaluated the impact of vitamin D levels in treatment-naive genotype 1 patients and submitted to conventional double therapy (PEG-IFN + ribavirin) in a French cohort. No impact was observed between vitamin D levels and response to antiviral therapy [30]. Studies have also evaluated genetic polymorphisms related to vitamin D cascade in Thai population and have observed that polymorphism in the DHCR7 gene may be a predictive marker of response to dual therapy (PEG-IFN + ribavirin) in a patient with HCV genotype 1 [31].

Egypt has the highest prevalence rate of HCV infection in the world, where hepatitis C is considered a major health problem. The standard treatment of HCV is combination therapy of PEG-IFN and ribavirin where SVR is only achieved in 30% of the patients. Due mainly to the adverse effects and cost of treatment, discontinuation of treatment is an important approach. In this way, Abdelsalam et al. [32] evaluated the association between vitamin D concentration and VDR polymorphisms with SVR acquisition, where the concentration of vitamin D, FokI and TaqI was considered as predictors for the antiviral response with the combination of pegylated interferon and ribavirin.

2. HCV disease progression in patients with metabolic alterations

Recently, epidemiological, clinical, and experimental studies have related HCV to liver steatosis and several metabolic derangements [33–35]. There is also evidence that HCV infection can induce IR through different mechanisms [34]. Insulin metabolism is affected by HCV directly

and indirectly leading to the production of several proinflammatory cytokines. The process of replication, assembly, and release of HCV from hepatocytes depend on close interactions with lipid droplets and host lipoproteins. The role of HCV in lipid metabolism of hepatocytes can lead to hepatic steatosis, especially in HCV patients infected by genotype 3 [36].

In genotype-1 patients, liver steatosis is directly related to metabolic factors including IR [37]. The impact of IR on the progression of liver disease has been debated and many evidence suggest that patients who have IR have a worse prognosis concerning multiple disease outcomes including progression of hepatic fibrosis and development of hepatocellular carcinoma [37]. Before the era of DAA for HCV infection treatment, IR also had an impact on treatment response, which has now been overcome by the high efficacy of these drugs. However, even with DAA treatment, IR is improved after the achievement of SVR [33].

There are several studies that analyze the association of HCV infection with IR and a meta-analysis of 34 studies found a positive correlation between HCV infection and increased risk of DM2 in comparison to the general population in both retrospective and prospective studies [38].

Regarding the studies that evaluated response to HCV treatment with interferon-containing regimens, it was observed that attaining SVR was associated with the improvement of IR defined by a lower homeostatic model assessment (HOMA)-IR after treatment [39, 40]. In addition, among patients submitted to treatment, those with a lower HOMA-IR had a higher chance of SVR [41].

Many studies found an association between higher HOMA-IR and fibrosis as well as the association of hepatocellular carcinoma with IR. Petit et al. [42] studied 123 HCV infected patients to investigate the host and viral specific factors associated with diabetes mellitus and IR in chronic hepatitis C patients. In diabetic patients, a score F4 was one of the factors related to the presence of diabetes mellitus and in patients without diabetes the HOMA-IR of METAVIR F 0 and F1 patients was significantly different compared to F2 and F3/F4 patients. They concluded that IR in non-diabetic HCV-infected patients was related to grading of liver fibrosis and occurred already at an early stage during HCV infection [42].

Hickman et al. [43] hypothesized that host metabolic factors might be associated with increased body mass index (BMI) and might play a role in liver disease progression. Thus, they studied 160 HCV patients at the time of liver biopsy and collected their serum for the assessment of the levels of insulin, c-peptide and leptin. They found that insulin was independently associated to fibrosis (P = 0.046) but not inflammation (P = 0.83). In addition, serum leptin levels were not associated to stage of fibrosis. So, in HCV patients infected by any genotype, increasing circulating insulin levels may be a factor responsible for the association between BMI and fibrosis [43].

Cua et al. [44] confirmed the impact of IR on fibrosis where they found that increased steatosis was related to high viral load (p = 0.001) but was not related to fibrosis (p = 0.1) in HCV genotype 3 patients. In HCV genotype I, body mass index (p = 0.04) and HOMA-IR (p = 0.01) contributed directly to steatosis. HOMA-IR was independently associated to fibrosis for HCV genotype 1 (OR, 3.22; p = 0.02) and genotype 3 (OR, 3.17; p = 0.04). [44].

Petta et al. [45] aimed to assess whether increasing degrees of IR, up to overt diabetes, were associated to steatosis and higher stages of fibrosis in patients with CHC resulting from genotype 1 HCV. About 201 genotype −1 HCV-infected patients were evaluated by liver biopsy and anthropometric and metabolic measurements, including IR, by the HOMA-IR (nondiabetic patients were defined as insulin resistant if HOMA-IR was >2.7). They evaluated three different groups concerning IR profile: 96 patients were noninsulin resistant (group 1), 76 were insulin resistant without diabetes (group 2), and 29 were diabetic (group 3). At multivariate analysis, fibrosis of >/=3 was independently associated with high necroinflammatory activity, low platelets, low cholesterol, high ferritin, and a high prevalence of IR. Diabetic patients were twice as likely to have severe fibrosis (60%) than those with IR but no diabetes (30%) ($p = 0.006$). This study concluded that in genotype 1 HCV infected patients, IR and overt diabetes are major determinants of advanced fibrosis, regardless of the degree of steatosis, mainly in the presence of severe necroinflammation.

Mohammed et al. [46] also concluded in a study that evaluated HCV infected patients compared to control group of non-infected HCV patients that IR may increase the rate of fibrosis progression in non-diabetic patients with chronic HCV. They suggested that follow up of hyperinsulinemia by serial assessment of HOMA-IR in non-diabetic HCV infected patients may be a biochemical indicator for progression of liver fibrosis [46]. On the other hand, some studies found no association between insulin resistance and liver fibrosis, like the one from Carvalho et al. who concluded that patients with chronic hepatitis C have significant metabolic alterations (hyperadiponectinemia and high HOMA-IR values) that are independent of HCV viremia and liver fibrosis [47].

Another issue that deserves discussion is the association of HCC and IR. Although the exact delineated mechanism is not yet established, there are some evidences to emphasize the involvement of HCV induced chronic inflammation, oxidative stress, IR, endoplasmic reticulum stress, liver steatosis and liver fibrosis in the progression of HCV chronic disease to hepatocellular carcinoma [48]. Possibly, IR is only one step involved among a complex interplay among factors that lead to HCC development. The impact of IR on HCC development is possible related to the fact that HCV interferes with insulin signaling by degradation of insulin receptor substrate 1 (IRS-1) and IRS-2 by suppressor of cytokine signaling (SOCS) protein or PI3K/Akt/mTOR pathway. IRS-1 is inactivated by TGF-α and PI3K/Akt also [49]. Based on these facts, the early stage of chronic HCV infection with increasing steatosis and IR creates an environment to develop hepatocarcinogenesis.

To investigate the role of IR and serum adiponectin level in hepatocellular carcinoma associated with chronic hepatitis C. Hung et al. analyzed three groups of patients and found that diabetes mellitus was more prevalent among HCV patients (35.6%, n = 59) compared to those infected by hepatitis B virus (HBV; 12.7%, n = 63), and non-HBV, non-HCV patients (7.1%, n = 28). Among HCV patients, age, serum insulin, HOMA-IR, DM and male gender were independently associated with HCC. This result was similar even when diabetic individuals were excluded from the analysis [50].

Noteworthy, most studies that evaluated the association of IR and HCV fibrosis are transversal studies. Longitudinal studies evaluating truly fibrosis progression and HCC development

in patients with and without insulin resistance are needed to better understand this link. However, in the new DAA era one must reevaluate the impact of IR in fibrosis progression since the elimination of the virus per se will probably improve liver histology as well.

3. HCV treatment in patients with metabolic syndrome and vitamin D deficiency

HCV infection may contribute to hepatic steatosis and to metabolic syndrome, forming a positive feedback that may further increase steatosis and culminate in steatohepatitis and fibrosis. As HCV infection is considered a curable disease, fibrosis can regress in some patients after therapy response [51, 52]. However, based on data from IFN era, infected patients have comorbidities, as metabolic syndrome, that may prevent fibrosis regression, leading eventually to a continued liver damage, even after viral eradication.

Vitamin D is an important physiological regulator that contributes to various biological, immunological, and metabolic functions in liver diseases. Previous *in vitro* results indicated that 25-OH vitamin D appeared to be significant associated with treatment response, particularly in the aspect of the rapid virological response (RVR) [53], which is an important predictive factor for SVR achievement [54, 55]. Patients with RVR have an approximately 90% of chance of treatment success after receiving PegIFN/RBV combined therapy, regardless of the viral genotypes [56–58]. The achievement of this early goal provides greater flexibility for tailoring the treatment duration on an individual basis and enhances the cost-effectiveness of treatment [55]. However, the impact of 25-OH vitamin D deficiency on RVR and the precise mechanisms underlying the inhibition of HCV replication were not thoroughly elucidated.

Some cross-sectional studies have shown associations between a higher 25-OH vitamin D level and response to therapy with PEG-IFN and ribavirin [15, 59, 60], while low levels are associated with poor response and its supplementation improves SVR rates. On the other hand, studies conducted in French HCV patients did not observed an impact of vitamin D levels in response to double therapy [30]. No causality can be established by cross-sectional studies and discordant results have been observed [61]. Associations may occur because healthier people are more exposure to sunlight and perform more physical exercises, which lead to a higher 25-OH vitamin D level. Also, chronic inflammation can shorten the half-life of 25-OH vitamin D and hepatic production of vitamin D-binding protein is reduced in patients with advanced liver disease and this may accelerate vitamin D turnover. Indeed, some studies pointed that its level before antiviral therapy has no impact on the efficacy of antiviral therapy, regardless the genotype [62]. On the other hand, an Italian study found high frequency of vitamin D deficiency among decompensated cirrhosis showing that vitamin D may play a role in the development of infections in patients affected by liver cirrhosis [63].

Potential relationship of vitamin D gene pathway has been suggested in the pathophysiology of HCV infection. Studies conducted in Asian and Latin America population did not find an association of VDR gene polymorphism to SVR in double therapy [28, 64]. On the other, studies conducted among European patients infected by genotypes 1, 2 and 3 found that VDR gene polymorphisms are independently related to the response to Peg-IFN + RBV therapy in CHC. These differences could be related to genetic differences among these studies [65, 66].

Few studies have evaluated the impact of vitamin D metabolism in therapy with direct-acting antivirals (DAAs). Recently, Cusato et al. [67] evaluated the impact of polymorphisms in genes (*CYP27B1*, *CYP24A1*, *VDBP* and *VDR*) related to vitamin D pathway on sofosbuvir and GS-331007 plasma levels in HCV mono-infected patients at 1 month of treatment. They found that genetic polymorphisms involved in vitamin D pathway influenced drug concentration. In future, it might be useful to understand if these polymorphisms can affect other DAAs concentrations; and to understand their role in the prediction of clinical variables, such as the probability to develop hepatocarcinoma or to influence the viral load decay.

High rates of early tumor recurrence were recently reported after therapy with DAAs in 103 HCV-infected patients with prior HCC [68]. Despite therapy with DAAs, the occurrence of liver cancer could not be reduced in cirrhotic patients with SVR [69]. Recently, a study conducted in Italy found an association of HCC risk factors to age, ribavirin administration, IL28B rs12979860 CC and previous treatments; VDR FokI CC, sex and insulin resistance were protective factors [67]. However, three distinct prospective cohorts showed no increased risk of HCC recurrence in 267 patients after DAA treatments [70]. Whether DAA treatments increase HCC occurrence or recurrence rates will remain a subject for debate until have emerged with a proper control arm to assess this important question [71].

With the efficacious DAAs regimens, comorbidities appear not to impair SVR. Long-term studies in very large patient cohorts treated with DAAs will elucidate the degree to which steatosis, steatohepatitis, and/or fibrosis reverse with SVR. The persistence of these comorbidities may prevent complete return to health in HCV-cured patients.

4. Conclusion

In conclusion, several metabolic alterations, such as, insulin resistance and DM2 have been observed among CHC patients. During double therapy with PEG-IFN and ribavirin, IR and vitamin D levels were important to define high successful rates of virological response. Nowadays, with the advent of DAAs, the rate of SVR have been increased, however high rates of early tumor recurrence were recently reported after therapy with DAAs. In addition, the role of vitamin D levels and genetic polymorphisms involved in vitamin D metabolism could be important predictors for viral response and evolution of clinical cases. Further studies should be necessary to confirm the impact of these factors during new antiviral regimens.

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Conflict of interest

The authors declare no conflict of interest.

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References

[1] World Health Organization (WHO). [Internet]. 2017. Available from: http://www.who.int/mediacentre/factsheets/fs164/en/. [Accessed: March 22, 2018]

[2] CDC. Hepatitis C Information for Health Professionals. Atlanta, GA, USA: Department of Health and Human Services, CDC; 2008. Available online: http://www.cdc.gov/hepatitis/HCV/index.htm (Accessed on)

[3] Wong RJ, Gish RG. Metabolic manifestations and complications associated with chronic hepatitis C virus infection. Journal of Gastroenterology and Hepatology. 2016;**12**:293-299

[4] Cannon CP. Mixed dyslipidemia, metabolic syndrome, diabetes mellitus, and cardiovascular disease: Clinical implications. The American Journal of Cardiology. 2008;**102**:5-9. DOI: 10.1016/j.amjcard.2008.09.067

[5] Lavanchy D. The global burden of hepatitis C. Liver International. 2009;**29**:74-81. DOI: 10.1111/j.1478-3231.2008.01934.x

[6] Alter MJ, Mast EE, Moyer LA, Margolis HS. Hepatitis C. Infectious Disease Clinics of North America. 1998;**12**:13-26

[7] Parvaiz F, Manzoor S, Tariq H, Javed F, Fatima K, Qadri I. Hepatitis C virus infection: Molecular pathways to insulin resistance. Virology Journal. 2011;**8**:474. DOI: 10.1186/1743-422X-8-474

[8] Hung CH, Lee CM, Lu SN. Hepatitis C virus associated insulin resistance: Pathogenic mechanisms and clinical implications. Expert Review of Anti-Infective Therapy. 2011;**9**:525-533. DOI: 10.1586/eri.11.33

[9] Fraser GM, Harman I, Meller N, Niv Y, Porath A. Diabetes mellitus is associated with chronic hepatitis C but not chronic hepatitis B infection. Israel Journal of Medical Sciences. 1996;**32**:526-530

[10] Serfaty L, Capeau J. Hepatitis C, insulin resistance and diabetes: Clinical and pathogenic data. Liver International. 2009 Mar;**29**(Suppl 2):13-25. DOI: 10.1111/j.1478-3231.2008.01952.x. Review. PubMed PMID: 19187069

[11] Akuta N, Suzuki F, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, et al. Amino acid substitutions in the hepatitis C virus core region of genotype 1b are the important predictor of severe insulin resistance in patients without cirrhosis and diabetes mellitus. Journal of Medical Virology. 2009;**81**:1032-1039. DOI: 10.1002/jmv.21473

[12] Scalioni LP, da Silva AP, Miguel JC, Espírito Santo MPD, Marques VA, Brandão-Mello CE, Villela-Nogueira CA, Lewis-Ximenez LL, Lampe E, Villar LM. Lack of association between hepatitis C virus core gene variation 70/91aa and insulin resistance. International Journal of Molecular Sciences. 2017 Jul 21;**18**(7). pii: E1444. DOI: 10.3390/ijms18071444

[13] Akuta N, Suzuki F, Hirakawa M, Kawamura Y, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Saitoh S, et al. Amino acid substitutions in the hepatitis C virus core region are the important predictor of hepatocarcinogenesis. Hepatology. 2007;**46**:1357-1364. DOI: 10.1002/jmv.22094

[14] El-Shamy A, Shindo M, Shoji I, Deng L, Okuno T, Hotta H. Polymorphisms of the core, NS3, and NS5A proteins of hepatitis C virus genotype 1b associate with development of hepatocellular carcinoma. Hepatology. 2013;**58**:555-563. DOI: 10.1002/hep.26205

[15] Petta S, Cammà C, Scazzone C, Tripodo C, Di Marco V, Bono A, Cabibi D, et al. Low vitamin D serum level is related to severe fibrosis and low responsiveness to interferon-based therapy in genotype 1 chronic hepatitis C. 15. Hepatology. 2010;**51**:1158-1167

[16] Steinvil A, Leshem-Rubinow E, Berliner S, Justo D, Finn T, Ish-shalom M, Birati EI, et al. Vitamin D deficiency prevalence and cardiovascular risk in Israel. European Journal of Clinical Investigation. 2011;**41**:263-268

[17] Fleet JC, DeSmet M, Johnson R, Li Y. Vitamin D and cancer: A review of molecular mechanisms. The Biochemical Journal. 2012;**441**:61-76

[18] O'Brien MA, Jackson MW. Vitamin D and the immune system: Beyond rickets. Veterinary Journal. 2012;**194**:27-33

[19] Sung CC, Liao MT, Lu KC, Wu CC. Role of vitamin d in insulin resistance. Journal of Biomedicine & Biotechnology. 2012;**2012**:634195

[20] Rosen CJ. Vitamin D and falls--are intermittent, high doses better? Nature Reviews. Endocrinology. 2011 Nov 1;**7**(12):695-696. DOI: 10.1038/nrendo.2011.185

[21] Bitetto D, Fabris C, Falleti E, Toniutto P. Vitamin D deficiency and HCV chronic infection: What comes first? Journal of Hepatology. 2011 Oct;**55**(4):944-5; author reply 945. DOI: 10.1016/j.jhep.2011.01.044

[22] Melo-Villar L, Lampe E, de Almeida AJ, de P Scalioni L, Lewis-Ximenez LL, Miguel JC, Del Campo JA, Ranchal I, Villela-Nogueira CA, Romero-Gomez M. Hypovitaminosis D and its relation to demographic and laboratory data among hepatitis C patients. Annals of Hepatology. 2015 Jul-Aug;**14**(4):457-463

[23] Baur K, Mertens JC, Schmitt J, Iwata R, Stieger B, Frei P, Seifert B, Bischoff Ferrari HA, von Eckardstein A, Müllhaupt B, Geier A, Swiss Hepatitis C Cohort Study Group.

The vitamin D receptor gene bAt (CCA) haplotype impairs the response to pegylated-interferon/ribavirin-based therapy in chronic hepatitis C patients. Antiviral Therapy. 2012;**17**(3):541-547. DOI: 10.3851/IMP2018

[24] Lange CM, Bojunga J, Ramos-Lopez E, von Wagner M, Hassler A, Vermehren J, Herrmann E, Badenhoop K, Zeuzem S, Sarrazin C. Vitamin D deficiency and a CYP27B1-1260 promoter polymorphism are associated with chronic hepatitis C and poor response to interferon-alfa based therapy. Journal of Hepatology. 2011 May;**54**(5):887-893. DOI: 10.1016/j.jhep.2010.08.036

[25] Ladero JM, Torrejón MJ, Sánchez-Pobre P, Suárez A, Cuenca F, de la Orden V, Devesa MJ, Rodrigo M, Estrada V, López-Alonso G, Agúndez JA. Vitamin D deficiency and vitamin D therapy in chronic hepatitis C. Annals of Hepatology. 2013 Mar-Apr;**12**(2):199-204

[26] Bitetto D, Fattovich G, Fabris C, Ceriani E, Falleti E, Fornasiere E, Pasino M, Ieluzzi D, Cussigh A, Cmet S, Pirisi M, Toniutto P. Complementary role of vitamin D deficiency and the interleukin-28B rs12979860 C/T polymorphism in predicting antiviral response in chronic hepatitis C. Hepatology. 2011 Apr;**53**(4):1118-1126. DOI: 10.1002/hep.24201

[27] Bitetto D, Fabris C, Fornasiere E, Pipan C, Fumolo E, Cussigh A, Bignulin S, Cmet S, Fontanini E, Falleti E, Martinella R, Pirisi M, Toniutto P. Vitamin D supplementation improves response to antiviral treatment for recurrent hepatitis C. Transplant International. 2011 Jan;**24**(1):43-50. DOI: 10.1111/j.1432-2277.2010.01141.x

[28] Scalioni LP, Santos BRD, Spritzer PM, Villela-Nogueira CA, Laura Lewis-Ximenez L, Pollo-Flores P, Bordalo Cathalá Esberard E, Brandão-Mello CE, Lampe E, Villar LM. Impact of vitamin D receptor and binding protein gene polymorphisms in clinical and laboratory data of HCV patients: Cross sectional study. Medicine (Baltimore). 2018 Feb;**97**(8):e9881. DOI: 10.1097/MD.0000000000009881

[29] Backstedt D, Pedersen M, Choi M, Seetharam A. 25-vitamin D levels in chronic hepatitis C infection: Association with cirrhosis and sustained virologic response. Annals of Gastroenterology. 2017;**30**(3):344-348. DOI: 10.20524/aog.2017.0120

[30] Belle A, Gizard E, Conroy G, Lopez A, Bouvier-Alias M, Rouanet S, Peyrin-Biroulet L, Pawlotsky JM, Bronowicki JP. 25-OH vitamin D level has no impact on the efficacy of antiviral therapy in naïve genotype 1 HCV-infected patients. United European Gastroenterology Journal. 2017 Feb;**5**(1):69-75. DOI: 10.1177/2050640616640157

[31] Thanapirom K, Suksawatamnuay S, Sukeepaisarnjaroen W, Tangkijvanich P, Treeprasertsuk S, Thaimai P, Wasitthankasem R, Poovorawan Y, Komolmit P. Vitamin D-related gene polymorphism predict treatment response to pegylated interferon-based therapy in Thai chronic hepatitis C patients. BMC Gastroenterology. 2017 Apr 17;**17**(1):54. DOI: 10.1186/s12876-017-0613-x

[32] Abdelsalam A, Rashed L, Salman T, Hammad L, Sabry D. Molecular assessment of vitamin D receptor polymorphism as a valid predictor to the response of interferon/ribavirin-based therapy in Egyptian patients with chronic hepatitis C. Journal of Digestive Diseases. 2016 Aug;**17**(8):547-553. DOI: 10.1111/1751-2980.12353

[33] Cheng FK, Torres DM, Harrison SA. Hepatitis C and lipid metabolism, hepatic steatosis, and NAFLD: Still important in the era of direct acting antiviral therapy? Journal of Viral Hepatitis. 2014;**21**:1-8. DOI: 10.1111/jvh.12172

[34] Milner KL, van der Poorten D, Trenell M, Jenkins AB, Xu A, Smythe G, et al. Chronic hepatitis C is associated with peripheral rather than hepatic insulin resistance. Gastroenterology. 2010;**138**:932-941. DOI: 10.1053/j.gastro. 2009.11.050

[35] Asselah T, Rubbia-Brandt L, Marcellin P, Negro F. Steatosis in chronic hepatitis C: Why does it really matter? Gut. 2006;**55**:123-130. DOI: 10.1136/gut.2005.069757

[36] Vanni E, Abate ML, Gentilcore E, Hickman I, Gambino R, Cassader M, et al. Sites and mechanisms of insulin resistance in nonobese, nondiabetic patients with chronic hepatitis C. Hepatology. 2009;**50**:697-706. DOI: 10.1002/hep.23031

[37] Kralj D, Virovi L, Juki C, Stojsavljevi S, Duvnjak M, Smoli M, Curci IB. Hepatitis C virus, insulin resistance, and steatosis. Journal of Clinical and Translational Hepatology. 2016;**4**:66-75

[38] White DL, Ratziu V, El-Serag HB. Hepatitis C infection and risk of diabetes: A systematic review and meta-analysis. Journal of Hepatology. 2008;**49**:831-844. DOI: 10.1016/j.jhep.2008.08.006

[39] Tanaka H1, Shiota G, Kawasaki H. Changes in glucose tolerance after interferon-alpha therapy in patients with chronic hepatitis C. Journal of Medicine. 1997;**28**:335-346

[40] Kawaguchi T, Ide T, Taniguchi E, Hirano E, Itou M, Sumie S, et al. Clearance of HCV improves insulin resistance, beta-cell function, and hepatic expression of insulin receptor substrate 1 and 2. The American Journal of Gastroenterology. 2007;**102**:570-576. DOI: 10.1111/j.1572-0241.2006.01038.x

[41] Poustchi H, Negro F, Hui J, Cua IH, Brandt LR, Kench JG, et al. Insulin resistance and response to therapy in patients infected with chronic hepatitis C virus genotypes 2 and 3. Journal of Hepatology. 2008;**48**:28-34. DOI: 10.1016/j.jhep. 2007.07.026

[42] Petit JM, Bour JB, Galland-Jos C, Minello A, Verges B, Guiguet M, et al. Risk factors for diabetes mellitus and early insulin resistance in chronic hepatitis C. Journal of Hepatology. 2001;**35**:279-283. DOI: 10.1016/S0168-8278(01)00143-X

[43] Hickman IJI, Powell EE, Prins JB, Clouston AD, Ash S, Purdie DM, et al. In overweight patients with chronic hepatitis C, circulating insulin is associated with hepatic fibrosis: Implications for therapy. Journal of Hepatology. 2003;**39**:1042-1048. DOI: 10.1016/S0168-8278(03)00463-X

[44] Cua IH, Hui JM, Kench JG, George J. Genotype-specific interactions of insulin resistance, steatosis, and fibrosis in chronic hepatitis C. Hepatology. 2008 Sep;**48**(3):723-731. DOI: 10.1002/hep.22392

[45] Petta S, Cammà C, Di Marco V, Alessi N, Cabibi D, Caldarella R, et al. Insulin resistance and diabetes increase fibrosis in the liver of patients with genotype 1 HCV infection. The American Journal of Gastroenterology. 2008;**103**:1136-1144. DOI: 10.1111/j.1572-0241.2008.01813.x

[46] Mohamed HR, Abdel-Azziz MY, Zalata KR, Abdel-Razik AM. Relation of insulin resistance and liver fibrosis progression in patients with chronic hepatitis C virus infection. International Journal of Health Sciences (Qassim). 2009 Jul;**3**(2):177-186

[47] Carvalho RF, Atta AM, de Oliveira IS, Santos TPS, Santos JPA, Schinoni MI, de Sousa-Atta MLB. Adiponectin levels and insulin resistance among patients with chronic hepatitis C. Acta Tropica. 2018 Feb;178:258-263. DOI: 10.1016/j.actatropica.2017.12.004

[48] Irshad M, Gupta P, Irshad K. Molecular basis of hepatocellular carcinoma induced by hepatitis C virus infection. World Journal of Hepatology. 2017 December 28;**9**(36):1305-1314. DOI: 10.4254/wjh.v9.i36.1305

[49] Aytug S, Reich D, Sapiro LE, Bernstein D, Begum N. Impaired IRS-1/PI3-kinase signaling in patients with HCV: A mechanism for increased prevalence of type 2 diabetes. Hepatology. 2003;**38**:1384-1392

[50] Hung CH, Wang JH, Hu TH, Chen CH, Chang KC, Yen YH, et al. Insulin resistance is associated with hepatocellular carcinoma in chronic hepatitis C infection. World Journal of Gastroenterology. 2010;**16**:2265-2271. DOI: 10.3748/wjg.v16.i18.2265

[51] Poynard T, Moussalli J, Munteanu M, Thabut D, Lebray P, Rudler M, et al. Slow regression of liver fibrosis presumed by repeated biomarkers after virological cure in patients with chronic hepatitis C. Journal of Hepatology. 2013;**59**(4):675-683

[52] Tachi Y, Hirai T, Miyata A, Ohara K, Iida T, Ishizu Y, et al. Progressive fibrosis significantly correlates with hepatocellular carcinoma in patients with a sustained virological response. Hepatology Research: The Official Journal of the Japan Society of Hepatology. 2015;**45**(2):238-246

[53] Huang JF, Ko YM, Huang CF, Yeh ML, Dai CY, Hsieh MH, Huang CI, Yang HL, Wang SC, Lin ZY, Chen SC, Yu ML, Chuang WL. 25-Hydroxy vitamin D suppresses hepatitis C virus replication and contributes to rapid virological response of treatment efficacy. Hepatology Research. 2017 Dec;**47**(13):1383-1389. DOI: 10.1111/hepr.12878

[54] Yu ML, Dai CY, Huang JF, et al. A randomised study of peginterferon and ribavirin for 16 versus 24 weeks in patients with genotype 2 chronic hepatitis C. Gut. 2007;**56**:553-559

[55] Yu ML, Chuang WL. Treatment of chronic hepatitis C in Asia: When east meets west. Journal of Gastroenterology and Hepatology. 2009;**24**:336-345

[56] Yu ML, Huang CF, Huang JF, et al. Role of interleukin-28B polymorphisms in the treatment of hepatitis C virus genotype 2 infection in Asian patients. Hepatology. 2011;**53**:7-13

[57] Zeuzem S, Hultcrantz R, Bourliere M, et al. Peginterferon α-2b plus ribavirin for treatment of chronic hepatitis C in previously untreated patients infected with HCV genotypes 2 or 3. Journal of Hepatology. 2004;**40**:993-999

[58] Huang CF, Yang JF, Huang JF, et al. Early identification of achieving a sustained virological response in chronic hepatitis C patients without a rapid virological response. Journal of Gastroenterology and Hepatology. 2010;**25**:758-765

[59] Gutierrez JA, Parikh N, Branch AD. Classical and emerging roles of vitamin D in hepatitis C virus infection. Seminars in Liver Disease. 2011;**31**:387-398

[60] Cholongitas E, Theocharidou E, Goulis J, et al. Review article: The extra-skeletal effects of vitamin D in chronic hepatitis C infection. Alimentary Pharmacology & Therapeutics. 2012;**35**:634-646

[61] Lange CM, Bibert S, Kutalik Z, et al. A genetic validation study reveals a role of vitamin D metabolism in the response to interferon-alfa-based therapy of chronic hepatitis C. PLoS One. 2012;**7**:e40159

[62] Kitson MT, Sarrazin C, Toniutto P, et al. Vitamin D level and sustained virologic response to interferon-based antiviral therapy in chronic hepatitis C: A systematic review and meta-analysis. Journal of Hepatology. 2014;**61**:1247-1252

[63] Buonomo AR, Zappulo E, Scotto R, Pinchera B, Perruolo G, Formisano P, Borgia G, Gentile I. Vitamin D deficiency is a risk factor for infections in patients affected by HCV-related liver cirrhosis. International Journal of Infectious Diseases. 2017 Oct;**63**:23-29. DOI: 10.1016/j.ijid.2017.07.026

[64] Hung CH, Hu TH, Lu SN, Chen CH, Wang JH, Lee CM. Association of vitamin D receptor gene polymorphisms with response to peginterferon plus ribavirin in asian patients with chronic hepatitis C. Journal of the Formosan Medical Association. 2016 Apr;**115**(4):278-283. DOI: 10.1016/j.jfma.2015.11.008

[65] García-Martín E, Agúndez JA, Maestro ML, Suárez A, Vidaurreta M, Martínez C, Fernández-Pérez C, Ortega L, Ladero JM. Influence of vitamin D-related gene polymorphisms (CYP27B and VDR) on the response to interferon/ribavirin therapy in chronic hepatitis C. PLoS One. 2013 Sep 20;**8**(9):e74764. DOI: 10.1371/journal.pone.0074764

[66] Cusato J, Allegra S, Boglione L, De Nicolò A, Baietto L, Cariti G, Di Perri G, D'Avolio A. Vitamin D pathway gene variants and HCV-2/3 therapy outcomes. Antiviral Therapy. 2015;**20**(3):335-341. DOI: 10.3851/IMP2853

[67] Cusato J, Boglione L, De Nicolò A, Favata F, Ariaudo A, Mornese Pinna S, Guido F, Avataneo V, Cantù M, Carcieri C, Cariti G, Di Perri G, D'Avolio A. Vitamin D pathway gene polymorphisms and hepatocellular carcinoma in chronic hepatitis C-affected patients treated with new drugs. Cancer Chemotherapy and Pharmacology 2018 Mar;**81**(3):615-620. DOI: 10.1007/s00280-018-3520-0

[68] Reig M, Mariño Z, Perelló C, Iñarrairaegui M, Ribeiro A, Lens S, Díaz A, Vilana R, Darnell A, Varela M, Sangro B, Calleja JL, Forns X, Bruix J. Unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing interferon-free therapy. Journal of Hepatology. 2016 Oct;**65**(4):719-726. DOI: 10.1016/j.jhep.2016.04.008

[69] Conti F, Buonfiglioli F, Scuteri A, Crespi C, Bolondi L, Caraceni P, Foschi FG, Lenzi M, Mazzella G, Verucchi G, Andreone P, Brillanti S. Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals. J Hepatol. 2016 Oct;65(4):727-733. doi: 10.1016/j.jhep.2016.06.015

[70] ANRS collaborative study group on hepatocellular carcinoma (ANRS CO22 HEPATHER, CO12 CirVir and CO23 CUPILT cohorts). Lack of evidence of an effect of direct-acting

antivirals on the recurrence of hepatocellular carcinoma: Data from three ANRS cohorts. Journal of Hepatology. 2016 Oct;**65**(4):734-740. DOI: 10.1016/j.jhep.2016.05.045

[71] Llovet JM, Villanueva A. Liver cancer: Effect of HCV clearance with direct-acting antiviral agents on HCC. Nature Reviews. Gastroenterology & Hepatology. 2016 Oct;**13**(10):561-562. DOI: 10.1038/nrgastro.2016.140

Chapter 7

Periodontal Implications of Hepatitis C Infection

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Additional information is available at the end of the chapter

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Abstract

Periodontal tissues exhibit important vascular, lymphatic, and nervous connections with the rest of the body. Thus, periodontal inflammation caused by the interaction between the subgingival bacterial biofilm and the host immune response has an impact reaching further than the oral cavity. The concept of "periodontal medicine" reunites the bidirectional relationships that exist between periodontal disease and systemic conditions such as diabetes mellitus or cardiovascular disease. The chronic inflammation of hepatic tissues during hepatitis C virus (HCV) infection causes changes in the general homeostasis that can reverberate at periodontal level and influence periodontal inflammation. Various mechanisms such as insulin resistance or pro-inflammatory cytokines production could be the link between the two conditions. In addition, periodontal inflammation could impact HCV transmission, as HCV RNA molecules and antibodies have been found in infected patients' saliva and gingival fluid. During periodontal inflammation, gingival bleeding is frequent, and the viral molecules could enter oral fluids while being carried by peripheral blood cells. Clinical particularities that suggest the onset of periodontal disease have also been frequently observed in HCV-infected patients. The connections between periodontal disease and hepatitis C need to take into consideration by practitioners of both specialties due to their important implications on clinical manifestations and treatment strategies.

Keywords: periodontal disease, hepatitis C, gingival crevicular fluid

1. Introduction

The concept of "periodontal disease" comprises a group of inflammatory conditions that address the supporting tissues (periodontium) of the tooth. If left untreated, periodontal disease will lead to alveolar bone loss and subsequent decreased tooth stability, eventually causing tooth loss [1].

The main cause or determinant factor for periodontal disease (PD) is the subgingival biofilm, composed of bacteria, fungi, and other microorganisms that are all bound together within a matrix [2]. The periodontal pathogenic bacteria are usually found in the oral cavity even in a healthy person, but under special circumstances (such as low immune response or other local and systemic favoring factors), they overgrow in number and become over-aggressive [3]. The normal immune response of the body can be altered by systemic diseases, while the tissular architecture and strength of the periodontal tissues is genetically determined, thus varying the way in which each patient reacts to periodontal bacteria aggression.

2. Periodontal structures

The periodontal structures that surround and support each tooth are represented by gingiva, periodontal ligaments, cementum, and alveolar bone [4]. These are divided into two separate categories: the superficial periodontium (composed of gingival and gingival fibers) and the profound periodontium (composed of periodontal ligament, cementum, and alveolar bone). The histological characteristics of the periodontal tissues are genetically determined, resulting in a variety of periodontal phenotypes that share common features but are also different in some aspects, especially in strength and resistance against bacterial assault. This explains why PD is more frequent in some individuals than in others, despite universal distribution of the periodontal pathogens [5].

The anatomical constituents of the periodontium are divided as follows (**Figure 1**):

1. Superficial periodontium:
    - gingiva: gingival epithelium + gingival connective tissue
    - gingival fibers
2. Profound periodontium/attachment apparatus:
    - periodontal ligament
    - cementum
    - alveolar bone

The gingiva protects and shelters the deeper structures of the periodontium (**Figure 1A**(a)). It is composed of a free, unattached area, called the marginal gingiva which surrounds each tooth,

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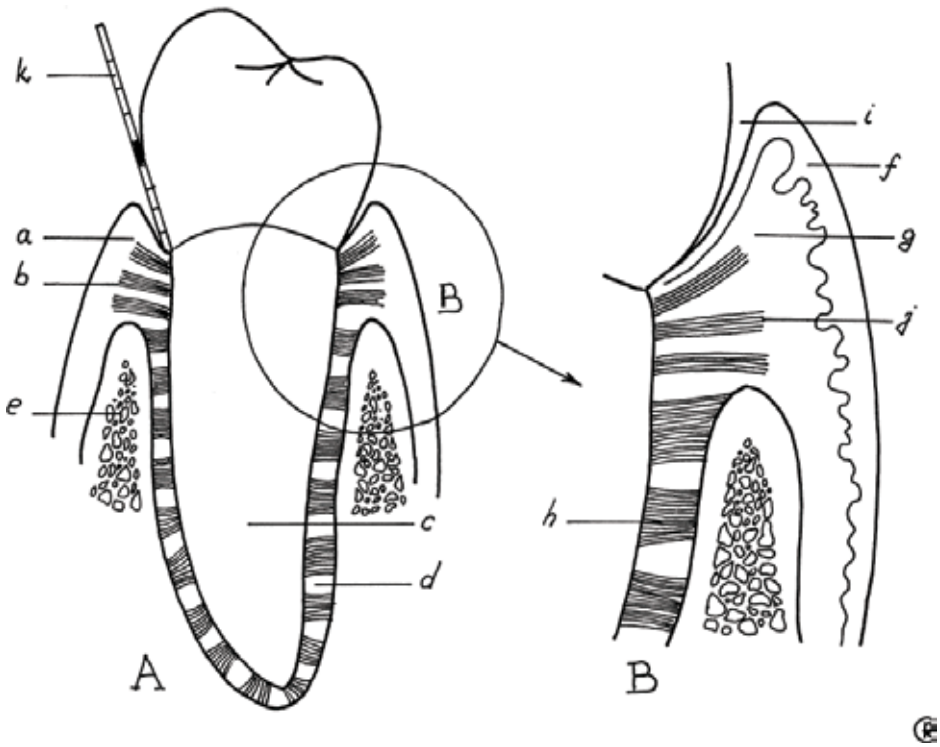

Figure 1. (A) The tooth and its surrounding periodontal structures: a, gingiva; b, gingival fibers; c, cementum; d, periodontal ligament; e, alveolar bone; k, periodontal probe. (B) Detail from (A): f, epithelium; g, connective tissue; h, periodontal space; i, gingival sulcus; j, junctional epithelium.

an interdental area called the gingival papilla and an attached area, called the attached gingiva, which is closely bounded to the underlying periosteum and the alveolar bone.

The gingival epithelium is made out of multiple cellular layers that are strongly keratinized where important mechanical forces are applied during chewing. The vascular and nutritional support of the epithelium is provided by the underlying connective tissue. Special structures called papillae rise from the connective tissue inside the epithelium, improving the adherence between the two and enhancing nutritional supply (**Figure 1B**(f,g)) [6].

Between tooth surface and the free gingiva, a narrow space called gingival sulcus is formed (**Figure 1B**(i)). In a healthy gingiva, this sulcus is only 0.5–2 or 3 mm deep. The periodontal probe is a special instrument used for assessing the depth of the gingival sulcus, an important indicator of PD presence and evolution (**Figure 1A**(k)). The gingiva becomes attached to the tooth surface in the vicinity of the enamel-cementum junction with the help of the junctional epithelium (**Figure 1B**(j)), a specialized type of tissue with less cellular layers than the rest of the gingival epithelium, thus allowing rapid changes of molecules between the gingival sulcus and the connective tissue. However, the thin architecture of the junctional epithelium makes it more liable to penetration by bacteria and their toxins [7, 8].

The gingival fibers are collagen fibers that are distinct from the periodontal ligament and are found inside the gingiva. They are disposed in a circular manner in the gingival connective tissue and keep the gingiva in close contact to the tooth (**Figure 1A**(b)) [6].

The cementum is a type of mineralized connective tissue that covers the roots of the teeth (**Figure 1A**(c)). In comparison to other mineralized connective tissues such as the enamel or the bone, the cementum has a lower concentration of mineral substances, therefore making it less durable and resistant. In addition to this, the average root cementum thickness is about 100 μm, making it predisposed to resorption. Nevertheless, the cementum can regenerate itself, as it is being stimulated by apposition forces like those used during orthodontic treatments and root planning [9].

The periodontal ligament fibers (**Figure 1A**(h)) are also made of collagen but these fibers are placed outside the gingiva, inside the narrow space between the root of the tooth and the alveolar bone called periodontal space (**Figure 1A**(d)). Therefore, they strongly connect the tooth inside its alveoli. Nevertheless, this connection is quite flexible and elastic, as it needs to adapt to various tooth movements that take place during chewing. Acting in a slingshot manner, the periodontal ligament fibers are anchored with one end in the root cementum and with the other in the alveolar bone, these ends of the fibers being called Sharpey fibers [4, 8].

The alveolar bone is composed of bony processes that extend from the base of the maxilla and the mandible, forming the sockets in which the teeth are placed (**Figure 1A**(e)). It is made out of spongious bone in its inside core and compact bone on the external and internal walls [8]. The alveolar bone attaches one end of the periodontal ligament fibers and distributes the masticatory forces from the teeth to the rest of the face and cranium. The existence of the bone is strongly related to the presence of teeth inside the sockets, as once the teeth are extracted the alveolar bone is quickly resorbed due to lack of stimulation by masticatory apposition forces [10].

3. Gingival crevicular fluid

When the periodontium is healthy, free of inflammatory conditions, the gingival connective tissue secretes a small quantity of clear, interstitial liquid inside the gingival sulcus, called the gingival crevicular fluid (GCF) [11]. The fluid is secreted as a result of different osmotic pressures between connective tissue vessels and the gingival sulcus content of bacteria [12]. When the subgingival bacterial plaque is matured and excessively grown, the fluid flow increases and changes its characteristics as periodontal inflammation unveils [11]. Thus, the gingival fluid will become rich in different types of cells such as epithelial, connective, bacterial, and host-immune cells.

The gingival fluid can be used for this reason as a mean to assess the severity and extent of PD and periodontal damage [11]. Moreover, the fluid contains a series of pro-inflammatory markers such as interleukins, enzymes (metalloproteinases (MMPs)), and growth factors that are all valuable indicators of PD activity. The quantitative and qualitative detection of these molecules in the gingival fluid is used as a method to assess the periodontal status during nonsurgical periodontal treatment [13], orthodontic therapy [14], and periodontal surgery [15].

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4. Periodontal disease

The classification of periodontal diseases has been the subject of extensive scientific debate. For simplification reasons, it can be considered that periodontal conditions are divided into inflammatory diseases of the superficial periodontium (gingivitis) and those of the profound periodontium (periodontitis). These two main types of periodontal conditions have extended subtypes and various clinical forms, but the bacteria-generated ones are the most common.

4.1. Gingivitis

The extended accumulation of bacterial plaque in the close proximity of the free gingival margin or inside the gingival sulcus will lead to the onset of an inflammatory process. The bacteria express toxins such as their lipopolysaccharide (LPS) that can trigger the immunological reaction of the body. As a result, gingival blood vessels become enlarged, lymphocytes are drawn to the site of the reaction, and more pro-inflammatory markers are secreted [16]. Although the inflammatory reaction is meant to stop the bacterial aggression, the uninterrupted bacterial growth in the biofilm will continue, leading to a chronic self-sustained inflammatory reaction. As the inflammation unveils, the gingiva will display the typical signs of inflammation: changing color from pink to first bright and then dark red, increasing volume due to edema and bleeding spontaneously or on chewing or periodontal probing. As long as the bacterial plaque is undisturbed by professional cleaning, the inflammation will proceed [17].

Some local factors such as crowded teeth, incorrectly adapted dental fillings, crowns or bridges, orthodontic appliances, or gingival recessions can result in an inappropriate oral hygiene, by creating niches from where the bacterial plaque is more difficult to remove [18]. In addition, the gingival status of the patient can be influenced by a series of systemic factors such as disease, medication, hormonal variations, or nutritional status that have an impact on the behavior of the gingival tissues when confronted even with a limited quantity of bacterial plaque. For example, medication used for hypertension treatment, epilepsy or immunosuppression can cause the gingival enlargement due to hyperplasia. The treatment of gingivitis consists of the professional removal of bacterial plaque and improving the favoring factors, either local or systemic. An important part of the treatment is represented by patient education and motivation for a well-kept oral hygiene. Gingivitis has a reversible character, and the gingival tissues return to their initial situation prior to the onset of the disease in most cases [16].

4.2. Periodontitis

When bacterial plaque is not removed, it will continue to grow and mature inside the gingival sulcus. More aggressive, Gram-negative anaerobic bacteria will begin to appear inside the biofilm, as the local conditions (low oxygen) enable them to thrive and multiply. These bacteria include important and highly aggressive periodontal pathogens such as *Porphyromonas gingivalis, Treponema denticola,* or *Tannerella forsythia.* As a result of the continuous bacterial aggression, the gingival junctional epithelium will begin to migrate in an apical direction, as an immune adaptation. Consequently, the gingival sulcus will become deeper, allowing the

extended growth of the biofilm and its colonization with aggressive periodontal pathogens [19]. The moment when the junctional epithelium migrates away from its normal position—the enamel-cementum junction, the gingival sulcus becomes a periodontal pocket and the damage caused by PD becomes irreversible. Consequently, the collagen fibers of the periodontal ligament and those inside the matrix of the alveolar bone are degraded by collagenase enzymes secreted by bacteria or by lymphocytes as a result of the chronic inflammatory reaction. In addition, as the alveolar bone is no longer stimulated by periodontal ligament fibers that transmit the masticatory forces, its resorption will be even more accelerated [20].

Further empowered by leukocyte- and monocyte-secreted pro-inflammatory markers (chemokines, interleukins, tumor necrosis factors (TNFs)), the periodontal inflammation can last even for years, with few clinical symptoms (mostly gingival bleeding, receding gums, increased tooth sensitivity) until the loss of periodontal ligaments and alveolar bone is so extensive that the teeth increase their mobility and eventually are lost. The rate of progression and severity of periodontitis can be influenced by systemic disease that impair the normal immune response of the body and favor the aggressive actions of the bacteria and their debilitating impact on the periodontal tissues [21]. The periodontal therapy aims to remove all subgingival biofilms and to partially reconstruct the lost alveolar bone with the aid of bone grafts in order to increase tooth stability. Nonsurgical periodontal treatment, systemic and local antibacterial medication, and periodontal surgery methods are used during active treatment, but the patient needs to be frequently recalled for reassessment, monitoring, and clean-up in order to prevent any relapse of PD [19].

5. Periodontal disease and systemic conditions

Periodontal conditions can impact the general well-being of the patient and can help to improve the outcome of other systemic or distant diseases through their treatment. Over the past decades, based on the results of clinical and fundamental studies, scientific research has elaborated a new concept, "periodontal medicine," which encompasses the bidirectional relationships that exist between periodontal diseases and some systemic conditions such as diabetes mellitus (DM), cardiovascular disease, or rheumatoid arthritis (RA) [22].

Diabetes mellitus associated to PD has received extensive and intensive research attention over time. Today, PD is officially recognized as the sixth complication of DM, alongside retinopathy, nephropathy, neuropathy, macrovascular disease, and modified wound healing [22]. The periodontal status of DM patients often includes overgrowth gingival tissues and the formation of gingival abscesses [23]. This chain of pathological events is strongly influenced by the glycemia level, as DM patients with well-controlled glycemia exhibit similar degrees of periodontal inflammation as non-DM ones [24]. Compared to healthy controls, DM patients exhibit an elevated risk for PD, by up to three times higher. DM history can also influence the onset of PD, as the major symptoms of periodontal destruction have been shown to manifest after 10 years of DM [25] or after the age of 30 for most patients [26].

Various mechanisms have been suggested in order to explain the impact of DM on periodontal status and PD onset and evolution. From a bacterial standpoint, it may seem that the increased glucose content of DM patients' saliva and GCF [27] could cause a change in the composition of the oral and periodontal microflora, favoring the development of periodontal pathogens such as *P. gingivalis* or *Aggregatibacter actinomycetemcomitans.* Another possible mechanism could reside in the impaired function of polymorphonuclear leukocytes (PMNs) that occur in diabetic patients. The cells' chemotactic, phagocytic, and adhesive capabilities are declined, facilitating debilitating defensive cellular mechanisms and periodontal pathogens infection of the periodontal tissues [28].

Pro-inflammatory markers play an important role in the onset, intensity, and extension of periodontal inflammation. In DM patients, significantly increased levels of pro-inflammatory cytokines produced by monocytes have been found [29]. This is believed to be caused by the hypersensitive monocyte/macrophage phenotype found in DM patients. This particular phenotype induces an overreaction to bacterial antigens, such as lipopolysaccharide (LPS). As a result, the production of cytokines is dramatically augmented [30]. For example, DM patients' monocytes produced 24–32 times higher quantities of TNF-alpha than non-DM monocytes, when stimulated by *P. gingivalis* LPS. Other cytokines are also produced in large amounts by DM monocytes subsequent to LPS stimulation: four times higher production of prostaglandin E2 (PGE2) and interleukin 1beta (IL-1beta) than non-DM monocytes [29]. When compared, the GCF levels of PGE2 and IL-1 beta were higher for DM patients'. Cytokines can activate the production of periodontal metalloproteinases (MMPs) (collagenase, gelatinase, elastase) [31] that subsequently impair the periodontal structures.

Coronary atherosclerosis disease (CAD) has been associated with dental infections [32], while ischemic heart disease has been correlated to the patient's history of missing teeth [33, 34]. Dental health was also found to be worse in myocardial infarction (MI) patients than in controls. Poor dental hygiene is also believed to increase twofold the risk of CAD. In addition, the Total Dental Index (TDI) can be considered as a predictor for CAD, according to some authors [35, 36].

In periodontal patients with more than 20% bone loss, the risk of CAD is increased by 50% [37]. Review articles of the existing literature on the subject mention advanced CAD risk in the presence of preceding PD, ranging from 14 to 222% [38]. The American Heart Association concludes that "periodontal disease is associated with atherosclerotic vascular disease independent of known confounders" [39], emphasizing that the direct causative correlation between PD and CAD has not yet been scientifically proven.

In the early stages of atheroma formation, monocyte cells adhere to the injured endothelial wall. The adhesion molecules that make this process possible are influenced by bacterial LPSs, prostaglandins, and pro-inflammatory cytokines. The increased levels of cytokines such as IL-1, TNF-alpha, and PGE2, which are found in periodontal disease's patients' serum, can facilitate the adhesion of the monocytes and therefore promote the formation of atheroma lesions. Periodontal therapy (scaling, root planning) has been shown to decrease the levels of pro-inflammatory cytokines and improve vascular health [40, 41]. The functional assessment of vascular endothelial function is also improved after periodontal therapy [40, 42].

Rheumatoid arthritis (RA) is a chronic inflammatory disease that leads to the dissolution of articular tissues (synovial membrane, bone, and cartilage). The disease is believed be an autoimmune dysfunction in which the body's own immune system begins to target articular tissue's cells.

The bone destruction is mediated by matrix degenerative enzymes such as metalloproteinases (MMPs), oversecreted by fibroblast and phagocytes cells during chronic inflammation. The secretion of these MMPs is normally inhibited, but the impairment of the immune system that takes place in RA and PD cancels the inhibitory action. Therefore, the collagen matrix of the bone tissue is degraded and bone loss occurs. As a result, bone decline can be indirectly assessed through MMPs detection in both RA and PD [43, 44].

Inflammatory conditions such as RA and PD imply the secretion of elevated levels of cytokines and other inflammatory mediators. Despite the vast range of pro-inflammatory cytokines, the cytokine profiles found in RA and PD patients share certain common molecules. For example, the prostaglandin E2 (PGE2) responsible for cell damage in RA also expresses elevated levels in PD, where alveolar bone breakdown is frequent [45]. Moreover, as *P. gingivalis* has been associated as a susceptible factor for rheumatoid arthritis, other cytokines such as IL-1, IL-6, or TNF-alpha exhibit inflated levels in both RA and PD and may also be responsible for the impairment of normal bone formation mechanisms, which eventually trigger tissue degradation [46, 47].

6. Chronic hepatitis C infection from an oral health perspective

Hepatitis C is a liver disease caused by the infection with the hepatitis C virus (HCV). Due to the increased number of infected individuals globally, it is considered to be a major public health issue, requiring important financial and scientific resources for the development of a universally available treatment [48]. The infection affects such a large number of people, because in its early stages, it can have no visible symptoms, making the spread of the virus easier from unaware infected persons to healthy ones. As the liver inflammation caused by the virus unveils, it becomes chronic in most patients, eluting the immune system's protective mechanisms [49]. Through the years, the chronic inflammation impairs the normal functionality of the liver, healthy hepatic tissue, and hepatocytes being replaced by reactive, fibrotic tissue. As a result, the complications of chronic hepatitis C will develop, the most frequent and dangerous ones being liver cirrhosis and hepatocellular carcinoma, both with harmful consequences for the patient [50].

An important feature of the HCV virus is that it can be easily transmitted. After entering the human body, the virus needs a cellular host in order to multiply. It does so mainly inside hepatocytes but it also targets peripheral blood cells, which means that viral RNA strands are found in infected patients' blood. Consequently, this makes blood and blood products the main medium of viral infection. Most frequently, this arises when using infected needles (for medical purposes or drug abuse) or after blood transfusions involving infected patients. The virus can also disseminate through unprotected sexual contact when one partner is infected, or by using poorly sterilized medical instrument [51]. The fact that the initial stages of HCV

infection develop without many visible or notable symptoms can facilitate the spread of the virus. Certain studies have found that viral RNA molecules can be found in saliva and gingival crevicular fluid as well, being carried by peripheral blood cells that enter those fluids [52]. As the viral molecules can be hosted inside peripheral blood cells, these can be easily transported at the gingival level especially during gingival inflammation, when the blood flow in the small capillary vessels is expanded. The gingival connective tissue position (underneath the thin gingival junctional epithelium) supports the transfer of these HCV-infected white cells into the gingival crevicular fluid, along with other bacterial and own damaged cells. During inflammation, the quantity of secreted gingival fluid is increased. In addition, inflamed gums bleed easily, facilitating the transfer of the HCV virus into the oral fluids such as saliva and gingival fluid [53, 54]. The infectious potential of these fluids is still debatable and requires further research.

As no vaccine for HCV has been developed, the main strategy for preventing the spread of the infection is the early detection of infected patients and the proper management of blood products originating from such subjects. Considering that the acute phase of infection is manifested only in a limited number of patients (one-tenth) and the symptoms are not necessarily common to those of other hepatic conditions (e.g., jaundice), the infected patient can easily oversee the infectious event [55]. This aspect claims the need for frequent blood testing (i.e., hepatic transaminases) in order to detect early signs of liver function impairment, or serum anti-HCV antibodies assessment. In young patients, the infection may be cleared up by the immune system, but in most cases, it becomes chronic and associated to other risk factors such as alcohol consumption, an unhealthy diet, and lifestyle being able to make the shift toward liver cirrhosis [56].

Extrahepatic manifestations of the disease can also occur in some patients, due to the fact that the virus can be hosted by the blood. As such, cryoglobulinemia, an inflammatory reaction caused by the increased amount of antibodies in the infected patients' blood, can develop, as the infected cells travel along blood vessels. The chronic hepatitis C infection generates a lot of stress on the patients' immune system. Under these circumstances, it has been noted that a series of autoimmune conditions can develop as a consequence of chronic hepatitis C, due to the impaired function of the immune system and the alteration of normal pro-inflammatory molecule production. Such autoimmune conditions include non-Hodgkin lymphoma, autoimmune thyroiditis, porphyria cutanea tarda, and possibly diabetes mellitus and rheumatoid arthritis [57]. The chronic hepatic inflammation caused by HCV can also impact the normal cellular intake of glucose, by causing insulin resistance [58]. Other non-autoimmune extrahepatic manifestations impact the normal function of the kidney, heart, and central nervous system [57, 59].

Nowadays, intensive efforts are made for the limitation of HCV infection spread. This entails raising public awareness about virus transmission and therefore testing for HCV. As the detection of viral antibodies or viral RNA in blood samples can be overcomplicated and unavailable in some situations, easier diagnosis tools such as salivary oral kits have been created [60]. These can improve the screening of the patients for infection and decrease the chance of involuntary transmission of the virus. However, such methods are not yet fully developed or universally accessible. The treatment of chronic hepatitis C complications is

expensive and complex, including antiviral medication and liver transplant. This further enhances the need for a smooth diagnostic tool, able to intercept the infection in its early stages, despite the lack of evident clinical symptoms. In the future, perfected and well-calibrated oral fluid test kits could provide a solution for widespread screening of the infection and a hope for its prevalence and transmission rates decline [61].

Some extrahepatic manifestations of HCV infection such as Sjögren syndrome and oral lichen planus can also impact the status of the oral cavity [62, 63]. In Sjögren syndrome, the secretion ability of the lachrymal and salivary glands is impaired. The decrease in salivary volume causes a chain of oral health issues, which include soreness and friability of the oral mucosa, an increased number of dental caries, and sialadenitis of the main salivary glands [64]. Oral lichen planus is a type of mucous lesion that is not painful and thus can be easily unobserved by the patient, especially if settled in the posterior areas of the oral cavity. This fact has clinical significance, as the lesion can have malignant potential and needs to be identified and treated as soon as possible [65]. Some cases of oral-squamous cell carcinoma have been reported in HCV patients, stating a correlation between the two diseases [66]. HCV-infected patients should be closely monitored in terms of periodontal health, as the combined effect of chronic inflammation, both hepatic and periodontal, could have a negative impact on their life quality and general life expectancy. The common immunological, clinical, and epidemiological implications of the two conditions should raise awareness for practitioners, by offering the possibility of better understanding of their pathogenic mechanisms and therapeutic opportunities.

7. Connections between HCV infection and periodontal status

HCV chronic infection can have important consequences on patient's life quality and influence the normal activity of defensive mechanisms, such as the host immune response. This can leave the patient vulnerable to the onset of other infections such as periodontal disease triggered by bacterial biofilm. Recent scientific data show that multiple-layered connections between HCV infection and periodontal disease could be possible, including transmission implications via oral fluids (saliva and gingival fluid), related clinical manifestations of the two conditions [63, 67–69], and common pathogenic pathways that may favor their evolution [68].

The proposed hypothesis claims that chronic hepatitis C can influence the onset of periodontal impairment. This connection could be possible through the HCV-mediated changes upon the patient's homeostasis. As it was recently suggested, one such mechanism could be insulin resistance [68]. Chronic inflammation or infections like hepatitis C have been shown to cause insulin resistance, meaning that cells become insensitive or unresponsive to insulin stimulation and thus with a decreased ability to absorb and metabolize the glucose [70]. Insulin resistance has also been noted in periodontal patients [71, 72], suggesting that preexisting HCV infection could create favorable conditions for the development of periodontal changes by altering the general cellular response to inflammation. Another explanation for the interaction between the two diseases and the way they are interconditioned is that the liver play an important role in the regulation of the immune response [73]. An impaired hepatic function caused by HCV

infection and its complications (liver fibrosis) can alter the general immune response by impaired neutrophil cell activity or overproduction of pro-inflammatory cytokines (interleukins, tumor necrosis factors) by the activated monocytes/macrophages. Such changes in the immune response have also been recorded during periodontal inflammation [74]. The similar immune response in the two conditions could suggest that, combined with the presence of subgingival bacterial biofilm, a preexisting inflammatory disease such as chronic hepatitis C could create the necessary immunological premises able to trigger the onset of periodontal damage in HCV patients [75, 76].

Similar pro-inflammatory cytokine profiles have been found in both chronic hepatitis C and periodontal disease. Various amounts of IL-1 (alpha and beta), IL-6, and TNF-alpha were found in HCV patients' serum [77, 78] as well as in periodontal patients [79]. A direct significant correlation emerged between serum levels of TNF-alpha, the degree of hepatic inflammation, and serum levels of adiponectin [80]. This association could further explain the impact of chronic hepatitis C on insulin resistance, since adiponectin serum levels can impact and correlate to insulin resistance and homeostasis glucose metabolism [80]. In saliva samples of periodontal patients, similar cytokine profiles to those in serum were found [81], while traces of viral RNA and anti-HCV antibodies were found in chronic hepatitis C patients' saliva [82, 83]. The gingival crevicular fluid exhibits similar characteristics to saliva in both cytokine profile in samples from periodontal disease sites [84] and HCV load with RNA molecules and antibodies [52, 53, 85]. As for other systemic periodontal comorbidities such as diabetes mellitus, cytokine profile plays an important role in the development of distant inflammation, mediated by pro-inflammatory markers triggered by other metabolic disease that impact the homeostasis [29].

The two most common hepatic enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), used for liver function assessment, can also be assessed in the gingival crevicular fluid and saliva. The level of these enzymes has been shown to fluctuate according to the inflammatory status of the periodontal tissues [86–88]. The fact that transaminases recorded a decreased activity subsequent to periodontal treatment suggests that the periodontal inflammation and the aggression caused by periodontal bacteria can have extending effects that exceed the periodontal tissues. Therefore, it could reach the liver and impact its normal function, being able to influence ongoing hepatic conditions such as chronic hepatitis. Thus, the relation between periodontal disease and HCV infection could be bidirectional, each of the two conditions having an impact upon the other one, both in terms of pathogenic mechanism, clinical manifestation, and therapeutic strategies.

The connection between HCV infection and periodontal disease is also suggested by some data that assesses its clinical impact on the manifestations of the two conditions. In a study comparing the periodontal status of patients with periodontal disease to those of patients with periodontal disease associating hepatitis C [89], the first group of patients exhibited a statistically significant larger number of remnant teeth than the second group. In addition, for the patients with periodontal disease and hepatitis C, the existing teeth were more frequently affected by periodontal pockets than the hepatitis-free patients, although not statistically significant. A statistically significant difference was recorded between the maximum periodontal

pocket depths of the groups, deeper periodontal pockets being found in HCV patients. Moreover, the gingival index (GI), assessing the degree of gingival inflammation, was also significantly higher for HCV and periodontitis patients than for hepatitis-free ones. A complementary study assessing the metabolic status of periodontal patients with or without comorbidities (chronic hepatitis and diabetes mellitus) [90] was set up in order to determine whether chronic hepatitis can impact the metabolic status in a similar manner that a well-studied and acknowledged periodontal comorbidity like diabetes mellitus does. The results showed that the metabolic status of HCV periodontal patients was different, similar to the changes that diabetes had caused on the metabolic status of periodontal patients. HCV patients could be more reluctant to seek dental treatment or could face a hesitant attitude among dental practitioners to perform complex surgical or rehabilitation treatments, due to the elevated risk of infection transmission. This opinion is enforced by a study showing that HCV patients exhibited a less favorable dental health status than a control group, with higher numbers of dental carries and missing teeth, but lower dental maneuvers such as fillings, crowns, or bridges [91].

As it has been previously suggested [68, 75, 76], the possible connection between periodontal disease and chronic hepatitis C becomes more than plausible. It seems that, given the holistic perspective on the pathogenic mechanisms of the human body, any disruption of its homeostasis caused by inflammatory processes ongoing even in distant territories like the periodontal and liver tissues has a general impact that reverberates across the entire biological structure and becomes relevant in terms of clinical manifestation. This aspect can help specialists to get a better understanding of the common pathophysiological mechanisms that govern these conditions and to provide better treatment strategies with a wider systemic perspective. Obviously, the matter still requires further scientific effort and research to provide comprehensive explanations to the issues that need to be addressed.

8. Conclusion

The periodontal implications of HCV impact the factors that favor the onset of periodontal inflammation such as the host immune response. The clinical manifestations of the periodontal disease (like gingival bleeding) influence the presence of the viral RNA molecules in the gingival fluid and saliva. Generally, hepatitis C patients can exhibit an impaired level of oral health and seek limited dental care, therefore creating additional premises for the onset of periodontal damage. These implications need to be further taken into consideration by both periodontists and hepatologists, in order to provide better oral health and health outcomes for their patients.

Conflict of interest

None.

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References

[1] Di Benedetto A, Gigante I, Colucci S, Grano M. Periodontal disease: Linking the primary inflammation to bone loss. Clinical & Developmental Immunology. 2013;**2013**:503754

[2] Zijnge V, Ammann T, Thurnheer T, Gmür R. Subgingival biofilm structure. Frontiers of Oral Biology. 2012;**15**:1-16

[3] Costalonga M, Herzberg MC. The oral microbiome and the immunobiology of periodontal disease and caries. Immunology Letters. 2014;**162**(2 Pt A):22-38

[4] Fiorellini JP, Stathopoulou PG. Anatomy of periodontium. In: Newman MG, Takei HH, Klokkevold PR, Carranza FA, editors. Carranza's Clinical Periodontology. 12th ed. Amsterdam: Elsevier; 2015. pp. 9-41

[5] Esfahrood ZR, Kadkhodazadeh M, Talebi Ardakani MR. Gingival biotype: A review. General Dentistry. 2013;**61**(4):14-17

[6] Nanci A. Periodontium. In: Nanci A, editor. Ten Cate's Oral Histology. 8th ed. Amsterdam: Elsevier; 2012. pp. 205-232

[7] Lindhe J, Karring T, Araújo M. Anatomy of periodontal tissues. In: Lang NP, Lindhe J, editors. Clinical Periodontology and Implant Dentistry. 6th ed. Hoboken: Wiley Blackwell; 2015. pp. 3-48

[8] Holmstrup P. Anatomy of periodontium. In: Wilson TG, Kornman KS, editors. Fundamentals of Periodontics. 2nd ed. Hanover Park: Quintessence; 1996. pp. 21-38

[9] Yamamoto T, Hasegawa T, Yamamoto T, Hongo H, Amizuka N. Histology of human cementum: Its structure, function, and development. The Japanese Dental Science Review. 2016;**52**(3):63-74

[10] Hienz SA, Paliwal S, Ivanovski S. Mechanisms of bone resorption in periodontitis. Journal of Immunology Research. 2015;**2015**:615486

[11] Barros SP, Williams R, Offenbacher S, Morelli T. Gingival crevicular fluid as a source of biomarkers for periodontitis. Periodontology 2000. 2016;**70**(1):53-64

[12] Griffiths GS. Formation, collection and significance of gingival crevice fluid. Periodontology 2000. 2003;**31**:32-42

[13] Duarte PM, da Rocha M, Sampaio E, Mestnik MJ, Feres M, Figueiredo LC, Bastos MF, Faveri M. Serum levels of cytokines in subjects with generalized chronic and aggressive periodontitis before and after non-surgical periodontal therapy: A pilot study. Journal of Periodontology. 2010;**81**(7):1056-1063

[14] Ren Y, Hazemeijer H, de Haan B, Qu N, de Vos P. Cytokine profiles in crevicular fluid during orthodontic tooth movement of short and long durations. Journal of Periodontology. 2007;**78**(3):453-458

[15] Eley BM, Cox SW. Cathepsin B/L-, elastase-, tryptase-, trypsin- and dipeptidyl peptidase IV-like activities in gingival crevicular fluid: A comparison of levels before and after periodontal surgery in chronic periodontitis patients. Journal of Periodontology. 1992 May;**63**(5):412-417

[16] Mariotti A. Plaque induced gingival disease. In: Lang NP, Lindhe J, editors. Clinical Periodontology and Implant Dentistry. 6th ed. Hoboken: Wiley Blackwell; 2015. pp. 366-376

[17] Fiorellini J, Stathpoulou P. Clinical features of gingivitis. In: Newman MG, Takei HH, Klokkevold PR, Carranza FA, editors. Carranza's Clinical Periodontology. 12th ed. Amsterdam: Elsevier; 2015. pp. 224-230

[18] Douglass CW. The epidemiology of periodontal diseases. In: Wilson TG, Kornman KS, editors. Fundamentals of Periodontics. 2nd ed. Hanover Park: Quintessence; 1996. pp. 13-21

[19] Kinane D, Lindhe J, Trombelli L. Chronic periodontitis. In: Lang NP, Lindhe J, editors. Clinical Periodontology and Implant Dentistry. 6th ed. Hoboken: Wiley Blackwell; 2015. pp. 381-390

[20] Dommisch H, Kebschull M. Chronic periodontitis. In: Newman MG, Takei HH, Klokkevold PR, Carranza FA, editors. Carranza's Clinical Periodontology. 12th ed. Amsterdam: Elsevier; 2015. pp. 313-320

[21] Ebersole JL. Immune responses in periodontal disease. In: Wilson TG, Kornman KS, editors. Fundamentals of Periodontics. 2nd ed. Hanover Park: Quintessence; 1996. pp. 111-144

[22] Mealey BL, Klokkevold PR. Impact of periodontal infection on systemic health. In: Newman MG, Takei HH, Klokkevold PR, Carranza FA, editors. Carranza's Clinical Periodontology. 12th ed. Amsterdam: Elsevier; 2015. pp. 202-213

[23] Schjetlein AL, Jørgensen ME, Lauritzen T, Pedersen ML. Periodontal status among patients with diabetes in Nuuk, Greenland. International Journal of Circumpolar Health. 2014;**73**:26093

[24] Arrieta-Blanco JJ, Bartolomé-Villar B, Jiménez-Martinez E, Saavedra-Vallejo P, Arrieta-Blanco FJ. Dental problems in patients with diabetes mellitus (II): Gingival index and periodontal disease. Medicina Oral. 2003;**8**(4):233-247

[25] Silvestre FJ, Miralles L, Llambes F, Bautista D, Solá-Izquierdo E, Hernández-Mijares A. Type 1 diabetes mellitus and periodontal disease: Relationship to different clinical variables. Medicina Oral, Patología Oral y Cirugía Bucal. 2009;**14**(4):E175-E179

[26] Kumar M, Mishra L, Mohanty R, Nayak R. Diabetes and gum disease: The diabolic duo. Diabetes and Metabolic Syndrome: Clinical Research and Reviews. 2014;**8**(4):255-258

[27] Casarin RC, Barbagallo A, Meulman T, Santos VR, Sallum EA, Nociti FH, Duarte PM, Casati MZ, Gonçalves RB. Subgingival biodiversity in subjects with uncontrolled type-2 diabetes and chronic periodontitis. Journal of Periodontal Research. 2013;**48**(1):30-36

[28] Alba-Loureiro TC, Munhoz CD, Martins JO, Cerchiaro GA, Scavone C, Curi R, Sannomiya P. Neutrophil function and metabolism in individuals with diabetes mellitus. Brazilian Journal of Medical and Biological Research. 2007;**40**(8):1037-1044

[29] Araya AV, Pavez V, Perez C, Gonzalez F, Columbo A, Aguirre A, Schiattino I, Aguillón JC. Ex vivo lipopolysaccharide (LPS)-induced TNF-alpha, IL-1beta, IL-6 and PGE2 secretion in whole blood from type 1 diabetes mellitus patients with or without aggressive periodontitis. European Cytokine Network. 2003;**14**(3):128-133

[30] Li Y, Lu Z, Zhang X, Yu H, Kirkwood KL, Lopes-Virella MF, Huang Y. Metabolic syndrome exacerbates inflammation and bone loss in periodontitis. Journal of Dental Research. 2015;**94**(2):362-370

[31] Franco C, Patricia HR, Timo S, Claudia B, Marcela H. Matrix metalloproteinases as regulators of periodontal inflammation. International Journal of Molecular Sciences. 2017;**18**(2):E440

[32] Mattila KJ, Valle MS, Nieminen MS, Valtonen VV, Hietaniemi KL. Dental infections and coronary atherosclerosis. Atherosclerosis. 1993;**103**(2):205-211

[33] Watt RG, Tsakos G, de Oliveira C, Hamer M. Tooth loss and cardiovascular disease mortality risk–results from the Scottish health survey. PLoS One 2012;**7**(2):e30797

[34] Asai K, Yamori M, Yamazaki T, Yamaguchi A, Takahashi K, Sekine A, Kosugi S, Matsuda F, Nakayama T, Bessho K, Nagahama Study Group. Tooth loss and atherosclerosis: The Nagahama study. Journal of Dental Research. 2015;**94**(Suppl 3):52S-58S

[35] Mattila KJ. Dental infections as a risk factor for acute myocardial infarction. European Heart Journal. 1993;**14**(Suppl K):51-53

[36] Zhang DH, Yuan QN, Zabala PM, Zhang F, Ngo L, Darby IB. Diabetic and cardiovascular risk in patients diagnosed with periodontitis. Australian Dental Journal. 2015;**60**(4):455-462

[37] Yu H, Qi LT, Liu LS, Wang XY, Zhang Y, Huo Y, Luan QX. Association of Carotid Intima-media thickness and atherosclerotic plaque with periodontal status. Journal of Dental Research. 2014;**93**(8):744-751

[38] Humphrey LL, Fu R, Buckley DI, Freeman M, Helfand M. Periodontal disease and coronary heart disease incidence: A systematic review and meta-analysis. Journal of General Internal Medicine. 2008;**23**(12):2079-2086

[39] Lockhart PB, Bolger AF, Papapanou PN, Osinbowale O, Trevisan M, Levison ME, Taubert KA, Newburger JW, Gornik HL, Gewitz MH, Wilson WR, Smith SC Jr, Baddour LM. Periodontal disease and atherosclerotic vascular disease: Does the evidence support an independent association?: A scientific statement from the American Heart Association. Circulation 2012;**125**(20):2520–2544

[40] Tonetti MS, D'Aiuto F, Nibali L, Donald A, Storry C, Parkar M, Suvan J, Hingorani AD, Vallance P, Deanfield J. Treatment of periodontitis and endothelial function. The New England Journal of Medicine. 2007;**356**(9):911-920

[41] Li C, Lv Z, Shi Z, Zhu Y, Wu Y, Li L, Iheozor-Ejiofor Z. Periodontal therapy for the management of cardiovascular disease in patients with chronic periodontitis. Cochrane Database of Systematic Reviews. 2017;**11**. DOI: CD009197

[42] Amar S, Gokce N, Morgan S, Loukideli M, Van Dyke TE, Vita JA. Periodontal disease is associated with brachial artery endothelial dysfunction and systemic inflammation. Arteriosclerosis, Thrombosis, and Vascular Biology. 2003;**23**(7):1245-1249

[43] Parks WC, Wilson CL, López-Boado YS. Matrix metalloproteinases as modulators of inflammation and innate immunity. Nature Reviews Immunology. 2004;**4**(8):617-629

[44] Silosi I, Cojocaru M, Foia L, Boldeanu MV, Petrescu F, Surlin P, Biciusca V. Significance of circulating and crevicular matrix metalloproteinase-9 in rheumatoid arthritis-chronic periodontitis association. Journal of Immunology Research. 2015;**2015**:218060

[45] Biyikoğlu B, Buduneli N, Kardeşler L, Aksu K, Oder G, Kütükçüler N. Evaluation of t-PA, PAI-2, IL-1beta and PGE(2) in gingival crevicular fluid of rheumatoid arthritis patients with periodontal disease. Journal of Clinical Periodontology. 2006;**33**(9):605-611

[46] Martu MA, Solomon SM, Sufaru IG, Jelihovschi I, Martu S, Rezus E, Surdu AE, Onea RM, Grecu GP, Foia L. Study on the prevalence of periodontopathogenic bacteria in serum and subgingival bacterial plaque in patients with rheumatoid arthritis. Revista de Chimie. 2017;**68**(8):1946-1949

[47] Shetty K. The role of salivary cytokines in the etiology and progression of periodontal disease. General Dentistry. 2006;**54**(2):140-143

[48] Petruzziello A, Marigliano S, Loquercio G, Cozzolino A, Cacciapuoti C. Global epidemiology of hepatitis C virus infection: An up-date of the distribution and circulation of hepatitis C virus genotypes. World Journal of Gastroenterology. 2016;**22**(34):7824-7840

[49] Ansaldi F, Orsi A, Sticchi L, Bruzzone B, Icardi G. Hepatitis C virus in the new era: Perspectives in epidemiology, prevention, diagnostics and predictors of response to therapy. World Journal of Gastroenterology. 2014;**20**(29):9633-9652

[50] Wilkins T, Akhtar M, Gititu E, Jalluri C, Ramirez J. Diagnosis and Management of Hepatitis C. American Family Physician. 2015;**91**(12):835-842

[51] Saito T, Ueno Y. Transmission of hepatitis C virus: Self-limiting hepatitis or chronic hepatitis? World Journal of Gastroenterology. 2013;**19**(41):6957-6961

[52] Suzuki T, Omata K, Satoh T, Miyasaka T, Arai C, Maeda M, Matsuno T, Miyamura T. Quantitative detection of hepatitis C virus (HCV) RNA in saliva and gingival crevicular fluid of HCV-infected patients. Journal of Clinical Microbiology. 2005;**43**(9):4413-4417

[53] Maticic M, Poljak M, Kramar B, Seme K, Brinovec V, Meglic-Volkar J, Zakotnik B, Skaleric U. Detection of hepatitis C virus RNA from gingival crevicular fluid and its relation to virus presence in saliva. Journal of Periodontology. 2001;**72**(1):11-16

[54] Açıkgöz G, Cengiz MI, Keskiner I, Açıkgöz S, Can M, Açıkgöz A. Correlation of hepatitis C antibody levels in gingival crevicular fluid and saliva of hepatitis C seropositive hemodialysis patients. International Journal of Dentistry. 2009;**2009**:247121

[55] Westbrook RH, Dusheiko G. Natural history of hepatitis C. Journal of Hepatology. 2014;**61** (Suppl 1):S58-S68

[56] Saludes V, González V, Planas R, Matas L, Ausina V, Martró E. Tools for the diagnosis of hepatitis C virus infection and hepatic fibrosis staging. World Journal of Gastroenterology. 2014;**20**(13):3431-3442

[57] Cacoub P, Gragnani L, Comarmond C, Zignego AL. Extrahepatic manifestations of chronic hepatitis C virus infection. Digestive and Liver Disease. 2014;**46**(Suppl 5):S165-S173

[58] Bose SK, Ray R. Hepatitis C virus infection and insulin resistance. World Journal of Diabetes. 2014;**5**(1):52-58

[59] Voulgaris T, Sevastianos VA. Atherosclerosis as extrahepatic manifestation of chronic infection with hepatitis C virus. Hepatitis Research and Treatment. 2016;**2016**:7629318

[60] Peeling RW, Boeras DI, Marinucci F, Easterbrook P. The future of viral hepatitis testing: Innovations in testing technologies and approaches. BMC Infectious Diseases. 2017;**17** (Suppl 1):699

[61] Hagan LM, Schinazi RF. Best strategies for global HCV eradication. Liver International. 2013;**33**(Suppl 1):68-79

[62] Carrozzo M, Scally K. Oral manifestations of hepatitis C virus infection. World Journal of Gastroenterology. 2014;**20**(24):7534-7543

[63] Alavian SM, Mahboobi N, Mahboobi N, Karayiannis P. Oral conditions associated with hepatitis C virus infection. Saudi Journal of Gastroenterology. 2013;**19**(6):245-251

[64] Wang Y, Dou H, Liu G, Yu L, Chen S, Min Y, Zhao K, Wang X, Hu C. Hepatitis C virus infection and the risk of Sjögren or sicca syndrome: A meta-analysis. Microbiology and Immunology. 2014;**58**(12):675-687

[65] Alaizari NA, Al-Maweri SA, Al-Shamiri HM, Tarakji B, Shugaa-Addin B. Hepatitis C virus infections in oral lichen planus: A systematic review and meta-analysis. Australian Dental Journal. 2016;**61**(3):282-287

[66] Nagao Y, Sata M. High incidence of multiple primary carcinomas in HCV-infected patients with oral squamous cell carcinoma. Medical Science Monitor. 2009;**15**(9):CR453-CR459

[67] Mahboobi N, Porter SR, Karayiannis P, Alavian SM. Oral fluid and hepatitis a, B and C: A literature review. Journal of Oral Pathology & Medicine. 2012;**41**(7):505-516

[68] Gheorghe DN, Foia L, Toma V, Surdu A, Herascu E, Popescu DM, Surlin P, Vere CC, Rogoveanu I. Hepatitis C infection and periodontal disease. Is there a common immunological link? Journal of Immunology Research. 2018:9. ID: 8720101

[69] Carrozzo M. Oral health in patients with hepatitis C virus infection: An underestimated problem? Oral Diseases. 2001;**7**:267-270

[70] Serfaty L, Capeau J. Hepatitis C, insulin resistance and diabetes: Clinical and pathogenic data. Liver International. 2009;**29**(2):13-25

[71] Nagao Y, Kawasaki K, Sata M. Insulin resistance and lichen planus in patients with HCV-infectious liver diseases. Journal of Gastroenterology and Hepatology. 2008;**23**(4):580-585

[72] Lim SG, Han K, Kim HA, Pyo SW, Cho YS, Kim KS, Yim HW, Lee WC, Park YG, Park YM. Association between insulin resistance and periodontitis in Korean adults. Journal of Clinical Periodontology. 2014;**41**(2):121-130

[73] Robinson MW, Harmon C, O'Farrelly C. Liver immunology and its role in inflammation and homeostasis. Cellular & Molecular Immunology. 2016;**13**(3):267-276

[74] Khan SA, Kong EF, Meiller TF, Jabra-Rizk MA. Periodontal diseases: Bug induced, host promoted. PLoS Pathogens. 2015;**11**(7):e1004952

[75] Han P, Sun D, Yang J. Interaction between periodontitis and liver diseases. Biomedical Reports. 2016;**5**:267-276

[76] Nagao Y, Kawahigashi Y, Sata M. Association of periodontal diseases and liver fibrosis in patients with HCV and/or HBV infection. Hepatitis Monthly. 2014;**14**(2):e23264

[77] Capone F, Guerriero E, Colonna G, Maio P, Mangia A, Castello G, Costantini S. Cytokinome profile evaluation in patients with hepatitis C virus infection. World Journal of Gastroenterology. 2014;**20**(28):9261-9269

[78] Sun QL, Ran W. Review of cytokine profiles in patients with hepatitis. World Journal of Gastroenterology. 2004;**10**(12):1709-1715

[79] Yücel OO. Inflammatory cytokines and the pathogenesis of periodontal disease. Immunome Research. 2015;**11**:093

[80] Hung CH, Lee CM, Chen CH, Hu TH, Jiang SR, Wang JH, Lu SN, Wang PW. Association of inflammatory and anti-inflammatory cytokines with insulin resistance in chronic hepatitis C. Liver International. 2015;**29**:1086-1093

[81] Jaedicke KM, Preshaw PM, Taylor JJ. Salivary cytokines as biomarkers of periodontal diseases. Periodontology 2000. 2016;**70**(1):164-183

[82] Caldeira PC, Oliveira e Silva KR, Silva TA, de Mattos Camargo Grossmann S, Teixeira R, Carmo MA. Correlation between salivary anti-HCV antibodies and HCV RNA in saliva and salivary glands of patients with chronic hepatitis C. Journal of Oral Pathology & Medicine 2013;**42**(3):222–228

[83] Xavier Santos RL, de Deus DM, de Almeida Lopes EP, Duarte Coêlho MR, de Castro JF. Evaluation of viral load in saliva from patients with chronic hepatitis C infection. Journal of Infection and Public Health 2015;**8**(5):474-480

[84] Fujita Y, Ito H, Sekino S. Correlations between pentraxin 3 or cytokine levels in gingival crevicular fluid and clinical parameters of chronic periodontitis. Odontology. 2012;**100**(2): 215-221

[85] Montebugnoli L, Dolci G. Anti-HCV antibodies are detectable in the gingival crevicular fluid of HCV positive subjects. Minerva Stomatologica. 2000;**49**(1–2):1-8

[86] Kudva P, Saini N, Kudva H, Saini V. To estimate salivary aspartate aminotransferase levels in chronic gingivitis and chronic periodontitis patients prior to and following non-surgical periodontal therapy: A clinico-biochemical study. Journal of Indian Society of Periodontology. 2014;**18**(1):53-58

[87] Persson GR, DeRouen TA, Page RC. Relationship between levels of aspartate aminotransferase in gingival crevicular fluid and gingival inflammation. Journal of Periodontal Research. 1990;**25**:17-12

[88] Persson GR, DeRouen TA, Page RA. Relationship between gingival crevicular fluid levels of aspartate aminotransferase and active tissue destruction in treated chronic periodontitis patients. Journal of Periodontal Research. 1990;**25**:81-87

[89] Gheorghe DN, Rusu D, Herascu E, Popescu DM, Surlin P, Rogoveanu I. Evaluation of liver chemistry tests and clinical parameters in patients with periodontal disease and chronic hepatitis C. Revista de Chimie (Bucharest). 2017;**68**(6):1252-1254

[90] Gheorghe DN, Surlin P, Herascu E, Vere CC, Cojocaru M, Iliescu AA, Rica AM, Popa S, Popescu DM. Metabolic status of periodontal patients with systemic diseases. Romanian Journal of Oral Rehabilitation. 2017;**9**(4):105-119

[91] Coates EA, Brennan D, Logan RM, Goss AN, Scopacasa B, Spencer AJ, Gorkic E. Hepatitis C infection and associated oral health problems. Australian Dental Journal. 2000;**45**(2): 108-114

Chapter 8

Hepatitis C: Host and Viral Factors Associated with Response to Therapy and Progression of Liver Fibrosis

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Abstract

The goal of this study was to identify the baseline host and viral factors of response to antiviral therapy in patients with chronic hepatitis C. Compared with interferon/ribavirin therapy, new current direct-acting antiviral (DAA) combination regimens significantly increased rate of sustained virologic response (SVR) and shorter treatment durations, but is still limited by viral resistance, adverse effects, and high cost especially in developing countries. Human genetic factors and heterogeneity within the HCV genome may be associated with virologic treatment failure before and after antiviral therapy. Further, HCV infection may contribute to the development of HCV-related liver disease and hepatocarcinogenesis, through modulating genetic and epigenetic state of certain genes implicated in control of critical cellular pathways. Previous results confirm the importance of host and viral factors and virus-induced genetic and epigenetic changes in predicting outcome and treatment response.

Keywords: hepatitis C virus, response to therapy, IFN-based antiviral therapies, direct-acting antiviral (DAA) agents, genetic variability, liver fibrosis, hepatocarcinogenesis, epigenetic changes

1. Introduction

Chronic hepatitis C virus (HCV) infection with the estimated worldwide prevalence of 1.1% is a global health problem, affecting 170 million people worldwide [1, 2]. About 15–45% of infected persons spontaneously clear the virus without any treatment, but remaining 60–80% will develop chronic HCV infection leading to fibrosis, cirrhosis and/or hepatocellular

carcinoma (HCC) [1, 3]. Previous results suggest that, worldwide, there were 1.75 million new HCV infections and annually an estimated 700,000 persons with chronic HCV die untreated [1]. Until 2014, the standard of care to treat HCV infection was pegylated interferon and ribavirin (PEG-IFN and RBV). However, first generation of (DAAs) NSA3/4A protease inhibitors (PI) were boceprevir and telaprevir, administrated with PEG-IFN and RBV [1]. These protease inhibitors were limited by viral resistance, adverse effect and long treatment duration and high cost especially in development countries [4]. The standard care of therapy is changing rapidly and new (DAAs) therapy such as sofosbuvir, daclatasvir, and the sofosbuvir/ledipasvir combination are part of the preferred regimens in the WHO guidelines with achieve cure rates above 95%. In comparison with older therapies, new therapy is more effective, safer, and better-tolerated with shorter treatment (usually 12 weeks), but high prices of the medicines limit the expansion of HCV in many countries [1]. However, challenges remain in optimizing current drug regimens, limiting the problem of resistance mutations and promote individual therapy [5–7]. Treatment predictors are important factors for management of therapy in patients with chronic hepatitis C infection.

1.1. Host and virus-related factors associated with response to therapy

Previous studies indicated that baseline host and virus related factors such genotype, viral load, age, gender, stage of liver fibrosis, and *IL28B* (interleukin-28B) polymorphisms were associated with therapy outcome [8–16]. Among viral factors, HCV genotype and baseline level of HCV-RNA are significant determinants of treatment outcome. Sustained virologic response (SVR) was defined as undetectable levels of HCV RNA, 24 weeks after cessation of treatment. Rapid virologic response (RVR) was defined as undetectable levels of HCV RNA at 4 weeks. Early virologic response (EVR) was defined as undetectable levels of HCV-RNA at week 12 (complete EVR) or ≥2 log reduction in HCV viral load from baseline (partial EVR), while non-response NR was defined as detection of serum HCV RNA 6 months after cessation of treatment. Thus, RVR is a strong predictor of SVR, but absence of an EVR is significant predictor for non-response (NR) to antiviral treatment. Moreover, viral kinetics during therapy provides information on how to individualize treatment [16, 17]. It is known that stages of liver fibrosis may be associated with response rates to PEG-IFN/RBV therapy. Further, patients with advanced liver fibrosis (METAVIR score F3-F4) were more frequently in non-responders (NR) than in patients with minimal or mild fibrosis (F0-F2), especially in patients with genotype 1 [8, 12, 18]. According to the previous results, infection with genotypes 2 or 3, younger ages, lower baseline viral load, and absence of advanced fibrosis were all strong predictors of SVR. Since 2011, therapeutic regimens for HCV genotype 1 patients were modified. Combination of NS3/4a protease inhibitors and pegylated interferon and ribavirin improved the SVR rates [19]. However, boceprevir- and telaprevir-based regimens are associated with side effect and lower efficacy than the newer DAA therapies. Also, this therapy is effective only in patients with genotype 1 [1, 20]. With respect to host and viral factors and first generation DAAs, viral kinetics is the most important predictive factor of SVR. The IL28B was associated with greater chances to shorten therapy but there is no correlation with SVR [8]. Second-generation DAAs have higher rates of SVR, are safer and can be used in combinations that obviate the need for interferon and ribavirin [1]. However, failure to new DAAs combinations is in association with patients with

poor response, genotypes 1a or 3, advanced liver cirrhosis, elevated level of viral load and the presence of human immunodeficiency virus (HIV) coinfection [21]. In contrast, some authors suggest that therapy outcomes are not significantly influenced by *IL28B* polymorphisms, HCV genotype, high baseline viral load, or prior interferon failure [22]. In the era of DAAs, surveillance of HCC after eradication of HCV by antivirus therapy is particularly important. Current new therapies with DAAs are associated with high rates of SVR, generally exceeding 90% even among patients with cirrhosis or prior treatment failure. Therefore, understanding of various host and viral factors associated with disease progression and development of HCC in chronic hepatitis C infection is important for implementing personalized treatment. One of the most interesting current questions concerns the impact of DAAs on HCC incidence [23].

1.2. HCV-host interactions and host genetic alterations

In the specific environment of every host, the outcomes of the HCV infection will be different. There are many factors which influence the therapy outcome and progression of liver disease. These factors include baseline clinical and pathohistological parameters. Also, host genetic landscape has effects on therapy outcomes and development of liver disease phenotypes. Genome-wide association studies (GWAS) have been created to track genetic polymorphisms in the human genome which associate with, virus clearance, therapy outcomes, and different stages of liver disease. Thus, this kind of the genome screening could be a useful tool in the diagnostics and the therapy of hepatitis C, and could lead to a personalized therapy.

1.2.1. Role of interferons (IFN) in HCV infection

HCV infection induces production of interferons λ (IFN-λ). IFN-λs bind to IFNL receptors (IFNLR) activating JAK-STAT signaling pathway which induces expression of *ISGs* (interferon-stimulated genes). The IFNLR is a heterodimer, consisting of two subunits, IL10R2 and IL28RA. There are four IFN-λs described so far: IFNL 1–4. The gene loci for these proteins are located on the chromosome 19. Study of GWAS has revealed polymorphisms in IFNL gene loci, which are in association with HCV clearance, either spontaneous or therapy-induced. Previously it was shown that single nucleotide polymorphisms (SNPs) near *IL28B* (rs12979869 and rs8099917) were strongly associated with response to PEG-IFN/RBV therapy in patients with genotype 1 and with spontaneous virus clearance [24–27]. In the neighborhood of *ILR3*, a novel dinucleotide polymorphism, ss469415590 (TT/ΔG), has recently been discovered which is in high linkage disequilibrium with rs12979869 and participate in formation of a novel gene *IFNL4*. The *IFNL4* gene creates IFNL4 protein, but TT variant of ss469415590 does not form the protein [28]. The TT variant has been shown to be beneficial to its carriers because it influences the spontaneous and therapy-induced virus clearance [26]. Among people of African ancestry, the polymorphism ss469415590 is in stronger association with virus clearance than the rs12979869 [28]. There are two variants of IFNL4 protein with impact on antiviral activity, which differ in only one amino acid at the place 70. The carriers of the variant with serine (P70S) have better therapy response and higher spontaneous virus clearance rate compared to carriers of the variant with proline. However, the expression of *ISGs* in the variant P70S is decreased, which is in discrepancy with its better antiviral activity. The researchers have

speculated this is probably due to decreased adaptive immunity as the consequence of high expression of *ISGs* in carriers of IFNL4-P70 variant [28]. The genes of human leukocyte antigen (HLA) family located on the chromosome 6 are associated with clearance of HCV infection. Some authors found that this connection is inconsistent, because of different systems of HLA typing, clinical phenotypes, and ethnic backgrounds. The only polymorphisms of *HLA* genes which are confirmed so far to have an association with virus clearance are HLA-DQB1*03 and HLA-DRB1*11 [29–32]. The receptors of natural killer (NK) cells Killer-cell immunoglobulin-like receptors (KIR) and their corresponding ligands, HLA class 1 proteins, also have a role in the immune response. Studies of the association of the polymorphisms of KIR and HLA-C and virus clearance have given controversial results. In some studies, the carriers of a KIR2DS3 (killer cell immunoglobulin like receptor, two Ig domains and short cytoplasmic tail 3) and homozygosity for *HLA-C1* were associated with spontaneous virus clearance [33, 34]. Polymorphisms of some other genes coding for the proteins which have a role in the immune response have also been shown to correlate with spontaneous/therapy-induced virus clearance. One of these genes is the gene for osteopontin which initiates T helper cells type 1 response, and has been shown to associate with the sustained viral response after the IFN therapy [35, 36]. Today, the role of the host genetic variability has been reduced due to high efficacy of new era interferon-free antiviral therapy. However, the high cost of this therapy limits its availability to the developed parts of the world.

1.2.2. The influence of host genetic alterations on the progression of liver fibrosis in chronic HCV infection

On the other hand, the host genetic variability has a significant role in the formation of different liver phenotypes. The phenotypes, such as fibrosis, steatosis, cirrhosis, or hepatocellular carcinoma (HCC) are the consequence of virus infection and disease progression. The polymorphisms of the immune response genes have effect on disease progression or inhibition. For example, the patients with chronic hepatitis C, which carry rs12979860CC variant, have higher fibrosis progression rate. This effect is even more pronounced in the younger females and in the carriers of the HCV type 3 [37]. The polymorphisms of apoptosis-related genes *MERTK* (MER proto-oncogene, tyrosine kinase), *TULP1* (tubby like protein 1) and *RNF* (ring finger proteins) gene family have association with the progression of the HCV-related fibrosis [38] and the genes of the major histocompatibility complex (MHC) with cirrhosis [39] and HCC [40]. However, these results need confirmation in the further research. Although having some potential, GWAS studies have many flaws. One of them is the impossibility of the screening of more extensive genetic changes, e.g., *copy number variation* (CNV) and epigenetic events, which also take part in the disease progression and the therapy response. Methodological failure is that these studies use conservative significance thresholds to eliminate false positive signals and many significant polymorphisms of low frequency could be unregistered, as reviewed in [41]. All discovered polymorphisms so far are currently not applicable to the clinical classification of the liver disease, because of their low predictive value. One of the possible solutions is the formation of the polygenic scores. For example, there is an earlier study which resulted in the formation of cirrhosis risk scores (CRS) based on the gene signature of seven genes [42]. In another study, a prediction model for liver fibrosis

was based on gene polymorphisms. This model includes polymorphisms of *IFNL* and clinical risk factors, which taken together give a risk for liver fibrosis [43]. Based on the fibrosis/cirrhosis risk score, therapy decisions can be made. Chronic inflammation and cirrhosis of hepatic cells lead to HCC. Hepatocellular carcinoma is the one of the deadliest type of cancer in the world. It represents a heterogeneous disease consisting of many tumor subpopulations with different frequencies of mutated genes. There are several genes which are affected, during the HCC progression, either by genetic or epigenetic alterations. In recent studies, the alterations of the *TERT* (telomerase reverse transcriptase), *CTNNB1* (catenin beta 1), *TP53* (tumor protein p53), and *AXIN1* (axin 1) genes are shown to correlate with tumor progression [44, 45]. *TERT* promoter mutations have been present in 64% of HCV-related HCC [44]. TERT is a subunit of the telomerase enzyme, whose mutations lead to telomere shortening and uncapping of chromosomes, which then leads to chromosome fusion and general chromosomal instability. *TERT* mutations and silencing of *CDKN2A* (cyclin dependent kinase inhibitor 2A) gene by promoter hypermethylation are the events that coincided [46]. It seems that *TERT* mutation is an early event in the cancerogenesis [45]. Frequent *CTNNB1* mutations have also been observed in HCV-related HCC [46]. There were attempts to classify HCC based on genetic signatures. The genetic signature represents one gene or a collection of genes, with characteristic expression profile, which is confirmed to be specific for the diagnosis, prognosis, and treatment response prediction [47]. Genetic signatures could be the means of classifying HCC in the groups which would facilitate diagnosis and therapy decisions. In the first attempt to make a molecular model for a diagnosis of an early HCC in the HCV patients, the best accuracy was achieved using the signature of three genes *LYVE1* (lymphatic vessel endothelial hyaluronan receptor 1), *GPC3* (glypican 3), and *BIRC5* (baculoviral IAP repeat containing 5). The *TERT* gene and *E-cadherin* were also shown to be informative in that model [48]. One European study revealed molecular signatures of 243 HCCs, based on fibrosis and cirrhosis score, and various risk factors, among which HCV was present in 26% of the cases. However, this study did not find any associations of these molecular signatures with HCV infection [45]. Cancer Genome Atlas Research found 26 frequently mutated genes in HCC. They identified *ALB* (albumin), *APOB* (apolipoprotein B), *LZTR1* (leucine zipper like transcription regulator 1), *EEF1A1* (eukaryotic translation elongation factor 1 alpha 1), *SMARCA4* (SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4), *AZIN1* (antizyme inhibitor 1), *RP1L1* (retinitis pigmentosa 1 like 1), *GPATCH4* (G-patch domain containing 4), *CREB3L3* (cAMP responsive element binding protein 3 like 3), *AHCTF1* (AT-hook containing transcription factor 1) and *HIST1H1c* (histone cluster 1 H1 family member c) gene mutations. Additionally, this study used methylation profiles, based on which the HCC were divided into four methylation clusters. The fourth cluster was characterized by high frequency of *CTNNB1* and *TERT* mutations, *CDKN2A* promoter hypermethylation and presence of HCV infection [46]. Besides smaller genetic changes, the changes which encompass bigger regions of DNA such as deletions, insertions, copy number variations, loss of heterozygosity also have a role in the HCC progression. All of these changes alongside hyper and hypomethylation lead to general chromosomal instability, which is prerequisite for tumor progression. There are examples of the direct influence of HCV on host genomic instability. HCV protein NS5A interacts with host gene *ASPM* (abnormal spindle microtubule assembly), which regulates mitotic spindle formation, causing interruption of the cell cycle, eventually

leading to chromosomal instability and HCC [49]. HCV Core protein induces polyploidy by decreasing of retinoblastoma-associated protein expression, thereby influencing mitotic cycle checkpoint, which also leads to chromosomal instability [50]. In addition, interaction of NS3/4A with serine protein kinase, which has a role in the cell cycle, leads to disabling cell repair system [51]. The polymorphisms in the host genome have effect on therapy response, virus clearance, and differentiation of HCV-induced liver phenotypes. The genetic signatures in the host genomes could be the means of therapy decisions making, and a way to personalize therapy. However, some other factors, such as epigenetic events and alterations of larger DNA regions, should also be taken into an account.

1.3. Impact of hepatitis C virus heterogeneity on therapy outcomes

The persistence of hepatitis C virus (HCV) infection and its poor susceptibility to treatment have been attributed, at least in part, to the high rate of genetic variability exhibited by the virus.

The persistence of HCV infection and its poor susceptibility to treatment is the consequence of high rate of virus genetic variability. Variability of virus is due to the low fidelity of viral RNA-dependent RNA-polymerase, which lacks proof-reading capacity, thus allowing the generation of quasispecies. Genetic variability is segregated within particular segments of the HCV genome, resulting in a number of highly variable regions [52]. These quasispecies play an important role in the escape from selective pressures by immune responses and antiviral therapies. A decrease or no change in the number of nucleotides substitutions in the protein kinase (PKR) binding domain (PKRBD) and the interferon-sensitivity-determining region (ISDR) were associated with failed to respond to PEG-IFN/RBV treatment [53]. It was reported that the presence of >4 mutations in the PKRBD region of NS5A protein was correlated with SVR to peg-IFN/RBV therapy [54]. Moreover, PKRBD sequences might be used as a prognostic guide for treating HCV-1-infected patients [54, 55]. Absence of substitutions at positions 70 and 91 in Core protein is a significant predictor for the success of IFN-based therapy [54]. On the other side, amino acid substitutions in the Core protein play an important role in early dynamics of viral replication during IFN-based therapy in chronic HCV infection and more frequent occurrence HCC. With respect to new DAAs therapy, efficacy of NS5A inhibitors can be blocked by presence of NS5A resistance-associated substitutions (RASs), but choice of DAA regimen and duration of therapy depends on multiple viral and host factors.

2. Epigenetic changes in HCV-induced liver diseases

Persistent infection with HCV is associated with the development of chronic liver diseases: fibrosis, cirrhosis and ultimately, HCC [56, 57]. Prevention of chronic hepatitis C and its complications is based on antiviral therapy and early detection of reliable molecular markers in persons under the risk [57, 58]. However, current antiviral therapies are not effective in many patients with chronic hepatitis C, so there is a need for a greater understanding of the factors leading

to progression to HCC in order to design novel approaches to prevention of HCV-associated complications [59]. Here, we discuss the current knowledge about the inter-relationship between HCV and pathophysiology of HCV-associated chronic liver diseases, with particular focus on the virus-induced host epigenetic changes leading to hepatocarcinogenesis.

2.1. Hepatocellular carcinoma

According to epidemiological evaluation, HCC is the fifth most common cancer and the second most common cause for cancer death in the world [60]. Although the prognosis of patients with HCC has marginally improved over the last few decades, the five-year survival rate remains poor as a result of late diagnosis. Consequently, the majority of patients with advanced HCC do not survive for longer than 6 months from the time of diagnosis [61]. It has been shown that in chronically infected patients, the risk of developing HCC is strictly correlated to fibrosis stage, and the incidence of HCC is more frequent in patients with liver cirrhosis than in those with mild fibrosis [62]. SVR to IFN-based therapies decreases HCC incidence in a large number of HCV patients, indicating the importance of eradicating the virus to prevent carcinogenesis [63, 64]. However, despite a successful virus clearance, the risk of HCC still exists in individuals with severe fibrosis and continuous HCC monitoring is recommended [65]. So, it is of great importance to determine molecular markers of progressive fibrosis, that could indicate the chronically HCV infected persons under the risk of developing HCC [66, 67]. HCV-induced hepatocarcinogenesis is a multifactorial process which results from a complex interaction among host, environmental, and viral factors [67]. It is considered that at least three host cellular pathways are affected in this process: cell cycle, proliferation, and apoptosis [68]. As HCV is an RNA virus with limited integration of its genetic material into the host's genome, it was first assumed that its ability to transform hepatocytes is linked to indirect mechanisms. Chronic inflammation induced by viral infection results in a permanent degenerative and regenerative processes and occurrence of progressive fibrosis and cirrhosis [69–71]. In addition, chronic inflammation leads to increased levels of reactive oxygen species (ROS), which damage hepatocytes and can lead to accumulation of genetic and epigenetic alterations in hepatic cells [67, 72]. All these events can promote neoplastic transformation of hepatocytes and the progression of malignant clones [73]. Later studies have shown that HCV is directly involved in hepatocarcinogenesis, through direct action of viral proteins on host tumor suppressors and proto-oncogenes [74, 75]. Several viral proteins have been shown *in vitro* to possess functions that could favor hepatocarcinogenesis, through inducing genetic and epigenetic alterations. In particular, the Core protein, NS3, NS4B and NS5A can transform various cell lines, either alone or in cooperation with oncogenes [76–80]. These proteins interact with a number of host factors and signaling pathways leading to the progression from chronic hepatitis C to liver cirrhosis and HCC [68].

2.1.1. Epigenetic changes in HCV-induced HCC

Many lines of evidence suggest that aberrant epigenetic changes associated with viral infection may trigger events that promote the neoplastic transformation of hepatocytes [59, 73]. Epigenetics is defined as heritable state of gene expression without altering DNA sequences. Epigenetic mechanisms include genomic DNA methylation, chemical modifications

of histone tails, and non-coding miRNA regulation [81]. Epigenetic changes play a critical role in control of cellular processes through switching genes on and off, thus leading to differential expression of proteins [82]. HCV infection has been shown to induce or correlate with some epigenetic changes that may contribute to HCV-related liver diseases, including hepatocarcinogenesis [68]. It has been proven that certain HCV-encoded proteins induce promoter methylation of multiple genes, thereby affecting their expression [83, 84].

2.1.2. Host genes promoter methylation induced by HCV

DNA methylation represents the addition of methyl group (CH_3) to a fifth carbon of cytosine residues within a CG dinucleotide, frequently referred to as cytosine-guanine dinucleotide (CpG). DNA methylation is an essential component of epigenetic machinery that regulates transcriptional state of many genes. Methylated promoters often lack transcriptional activity, which could result in gene inactivation. As a transcriptional regulator, DNA methylation has a considerable impact on the development of many cancers, including HCC [81, 85]. Aberrant promoter hypermethylation of tumor suppressor genes involved in the cell proliferation, apoptosis, cell adhesion, DNA repair, and detoxification is frequently detected in HCC, resulting in loss of the corresponding gene function [86, 87]. It is believed that changes in DNA methylation patterns are early events in hepatocarcinogenesis and they can even occur at the early stages of HCV induced liver fibrosis [88]. This is supported by the results of the study conducted by Zekri et al., in which they demonstrated that methylation of certain host genes increase with liver disease progression, from fibrosis to HCC [89]. Moreover, the same group of authors has been shown that methylation of certain tumor suppressor genes affect the response to the antiviral therapy [90]. Considering this, a better understanding of methylation changes and how they correlate with disease progression will help in finding novel biomarkers for early detection of HCC and its prevention.

2.1.3. HCV-encoded proteins inducing host genes methylation

It has been demonstrated that HCV Core protein up-regulates levels of DNA methyltransferase (DNMT) 1 and 3b and induces promoter hypermethylation of tumor suppressor genes like *p16* (*CDKN2A*) and *E-cadherin* [91, 92]. Consequent inhibition of p16 expression results in inactivation of pRb (retinoblastoma protein) and subsequent activation of E2F transcription factor 1 (E2F1), which lead to growth stimulation of hepatocytes. Inactivation of *p16* tumor suppressor gene, that regulates cell cycle, appears to play an important role in the pathogenesis of HCC. It has been demonstrated that reactivation of p16 by transferring the *p16* gene can inhibit the proliferation and reduce the invasive ability of HCC cells [93]. Down-regulation of *E-cadherin* by Core-induced hypermethylation leads to epithelial-mesenchymal transition, cell detachment from the surrounding matrix, and migration outside of the primary tumor site, which is known to be a critical event during the late stage of carcinogenesis [94]. Besides these, methylation of some other tumor suppressor genes, like suppressor of *SOCS-1* (cytokine signaling 1), *GSTP1* (glutathione S-transferase pi 1), *APC* (adenomatous polyposis coli), and *RASSF1A* (Ras association domain family member 1), has been detected in HCV-associated HCC compared normal liver [95, 96]. Abnormal promoter methylation of most of these genes

was detected in the plasma/serum DNA as well as in the tissue DNA of HCC patients, which gives opportunity for designing noninvasive blood tests for detection of methylation markers and to distinguish HCV patients who will eventually progress to advanced stages of fibrosis [97]. Zhang et al. reported that the analysis of methylation status of *RASSF1A*, *p16*, and *p15* (cyclin-dependent kinase inhibitor 2B, *CDKN2B*) in serum DNA of infected people could be a valuable biomarker for early detection of HCC in the populations at high risk, including chronic HCV infection [98]. Iyer et al. recorded high frequencies of *p15*, *p16*, *APC*, *FHIT* (fragile histidine triad), and *E-cadherin* promoter methylation in the plasma and liver tissue of HCV-associated HCC patients, with high concordance for all examined genes [99]. Zekri et al. have shown that methylation of *MGMT* (O-6-methylguanine-DNA methyltransferase) gene can be used as a predictor of response to the antiviral therapy, while *RASSF1A* methylation status could be a marker of fibrosis severity [90]. As authors reported, promoter methylation of *MGMT* gene appeared at higher frequencies in the NR than in the responders, which was explained by the fact that MGMT has an important role in protecting cells against DNA damage, via triggering DNA repair mechanisms [100]. On the other hand, the same group of authors has shown that *RASSF1A* methylation was significantly higher in HCV patients with mild fibrosis, which support the role of an intact *RASSF1A* gene in inducing the fibrogenesis in chronic HCV patients [90]. Another study by Hayashi et al. reported that HCC patients with SVR have different molecular alterations compared to NR with continuous HCV infection [101]. This group of authors observed lower frequencies of *p16*, *RB1* (RB transcriptional corepressor 1) and *PTEN* (phosphatase and tensin homolog) genes promoter hypermethylation in patients with SVR, while methylation of *p15* and *p14* (ARF tumor suppressor) genes was not detected in this group of patients, compared to those with the present HCV infection. Interestingly, *p16* methylation was detected with the highest frequency in the both groups, suggesting important role of p16 gene in the development of SVR-HCC. Authors speculated that *p16* in hepatic stem cells might be methylated in the continuous presence of HCV. These cells with methylated *p16* gene might survive and grow after eradication of HCV by IFN therapy. In addition, despite an improved understanding of mechanisms leading to HCV-induced HCC and development of highly potent antiviral therapy, HCV-related HCC remains a global health problem. The development of valuable molecular biomarkers will be of a great importance to distinguish a group of HCV infected people with a high risk for hepatocarcinogenesis. Based on the studies conducted so far, genomic DNA methylation could function as a non-invasive, sensitive, and specific biomarker for prediction the response to the antiviral therapy and early detection of HCC.

2.2. MicroRNA biogenesis and function

MicroRNA (miRNA) are non-coding genetic elements participating in the regulation of gene expression by RNA interference in plants and animals, while in human cells, are associated with viral infection, as well. According to The Encyclopedia of DNA Elements (ENCODE), approximately 75% of the human genome transcribes into a various types of RNA molecules, coding and non-coding [102, 103]. Among non-coding RNA (ncRNA), miRNAs emerged as biologically, physiologically, and clinically significant, considering the fact that they silence translation of partially complementary target messenger (mRNA) molecules. MicroRNAs participate in the regulation of gene expression by RNA interference. In the last 15 years,

miRNAs were linked with various types of diseases and disorders, and majority of investigations were based on the changes in their expression levels. MicroRNAs are encoded by a miRNA gene, which transcribes into an immature-primary microRNA (pri-miRNA) molecule, a double stranded, hairpin-like genetic structure. Then, two enzymes-RNase endonuclease III Drosha, and DGCR8 (molecular anchor part of a microprocessor complex), transform pri-miRNA into a 70 nucleotides (nt) long precursor microRNA (pre-miRNA) in the nucleus [104]. The process of miRNA maturation occurs in the cytoplasm, where Dicer or Argonaute (Ago), and other protein-partners, cooperators cleave pri-miRNA into a 22 nt long miRNA, ready to be recognized by RNA-induced silencing complex-RISC [105, 106]. The recognition and binding of miRNA "seed" sequence to 3′ untranslated region (3′UTR) of target mRNA incompletely complementary with "seed" region at miRNA molecule results in either translational repression, or mRNA degradation. Translational repression and degradation result in the decrease of protein levels, thus changing genetic, biological, and physiological processes, activity of various signaling pathways. So, main characteristic of miRNAs is to regulate amounts of synthesized proteins [107]. miRNAs are present in every human cell, and in body fluids (as circulating), such as serum, plasma, urine, saliva, and even gingival liquid. MicroRNA circulates through the body via body fluids as free-circulating miRNAs, and packed into the exosomes and vesicles, as exosomal miRNAs. Extracellular, exosomal circulating miRNAs may carry over the information about disease progression, infection status, and other clinic pathological parameters of HCV infection and HCC formation and progression, but it is still not completely clear. Liver-specific miRNAs such as miR-122 and miR-192, may be released from damaged liver cells, providing the information about liver. Exosomal and circulating miRNAs, as well as miRNAs extracted from tissue may be involved in intercellular communication. Besides the fact that nearly 300 miRNAs are expressed in normal, healthy liver, miR-122 represents the major fraction of liver-specific miRNAS, together with miR-192, miR-199a/b-3p, miR-101/99a, and members of oncogenic let-7 family [108].

2.2.1. MicroRNA in HCV infection and HCC

MicroRNAs are described as onco miRNAs, some of them as tumor suppressive, and several of them even have dual role in cancer pathogenesis and presumably in other physiological processes and pathological condition [109]. During the HCV infection, onco miRNAs such as miR-21/155/221 activate and might cause formation and facilitate progression of HCC. On the other hand, the decrease of some tumor suppressive miRNAs during the HCV infection might also cause hepatocarcinogenesis, such as miR-198 [110]. Firstly, some miRNAs directly interact with the genome of HCV. Secondly, several miRNAs are potential biomarkers of the presence or progression of the HCV infection. Thirdly, several miRNAs are indicators of HCC formation and/or progression. Fourthly, there are miRNAs associated with HCC genotype and clinicopathological characteristics of infected patients and histopathological characteristics of tumors, while some miRNAs such as miR-134/320c/483-5p may be used as early biomarkers for HCV infection in the future. Finally, some miRNAs, such as miR-122 represent potentially great targets for future therapeutics in the aspects of treatment of HCV-associated liver diseases and HCC. Large numbers of different studies have recently been focused on miRNAs in a different points related to different points of HCV infection, i.e., replication of the virus, viral

genotype, response to HCV therapy, lipid status, liver function indicators, stage of liver fibrosis, HCC grading, and response to chemotherapy. Changes in the expression levels of miRNA several micro RNAs such as miR-21/122/134/141/155/192/199/221/320c/373/483-5p/491/758, let-7b, etc., are associated with different stages of the viral life cycle and the progression of infection [111]. Circulating miRNAs, miR-122, and miR-222 have been shown to be valuable as potential future diagnostic tool for HCV infection within the Egyptian patients [112]. Upregulated miR-10a/15a/17-5p were associated with HCV-related HCC, miR-122 was characterized as potential diagnostic tool for HCC within HCV-infected individuals, while overexpression of miR-221/222-3p was characteristic exclusively for HCV-related HCC [108]. MicroRNA 122 is a liver-specific miRNA, a regulator of HCV tendency to infect hepatic cells. miR-122 is crucial for efficient HCV infection and viral spread and speed up the replication of the virus in hepatocytes, and facilitates viral protein synthesis [113]. Besides miR-122, as the mostly studied miRNA in HCV-HCC patients that recognizes two different sites at HCV 5'UTR, increased levels of miR-448 and miR-196 attenuate HCV replication by binding to the Core and NS5A sequences of the HCV genome [114]. Secondly, some miRNAs, such as miR-199a have opposing characteristics during the HCV infection. Namely, miR-199 targets sequences of HCV genome and blocks the transcription of HCV RNA [115]. Thirdly, during the HCV infection, miR-155 is usually up-regulated. Higher levels of the well-known onco-miRNA, miR-155 induce proliferation of hepatocytes, increasing the chance of HCC formation [116]. For example, changes in the expression levels of miRNAs such as miR-134/320c/483-5p were shown to be significantly higher within the HCV-infected individuals, compared with healthy controls. In our previous article, we described heterogeneity in behavior of microRNA in cancer studies [109, 117]. Another evidence is supporting our observation on the importance to notice how level changes of the particular miRNA can be important for one event, while having no significance for another similar event, related to the same type of the disease or pathological condition, showing high specificity of some miRNA molecules. Namely, miR-20a/92a levels investigated in the sera of the patients having HCV-related liver disease were associated with the disease severity, and the higher grade of liver fibrosis, while levels of the same miRNAs have not shown any association with grade of liver fibrosis and other pathological characteristics examined within the patients with non-HCV-related liver diseases. Another example of involvement of miRNAs in various segments of HCV-HCC pathology, HCV proteins change levels of miR-193a that results in reduction of sensitivity to chemotherapy of HCC patients [118]. Furthermore, in our article related to the heterogeneity of miRNAs. According to several researches, it has been shown that miR-122 may be anti-tumorigenic properties in mice knockdown studies [119].

2.2.2. MicroRNA as future therapeutics for HCV and HCC

Considering the fact that several miRNAs can modulate expression of up to a dozens or even hundreds of target genes, it is not surprising that miRNA-based therapy is still challenging. Nevertheless, this multi-targeting ability also represents an advantage for future miRNA-based therapeutics. There are two major approaches for future miRNA-related therapeutics miRNA inhibition (Lock nucleic acid (LNA) anti miRNAs, antago miRNAs, miRNA zippers, small molecules inhibitors of miRNAs, and miRNA sponges) and miRNA substitution strategies (miRNA mimics and miRNA vectors), with the purpose to either silence miRNA activity

or to replace absent miRNA molecules [109, 110]. Several studies proposing that miRNA panels could be used in near future as biomarkers for screening of the HCV-related liver diseases. The changes in their expression levels were associated with staging of liver disease progression and anti-HCV therapeutics [108]. Probably, manipulation with several miRNAs at the same time might be crucial in treatment of HCV-HCC. For example, inhibition of upregulated miRNAs involved in viral replication, such as miR-122, simultaneously with miRNAs, in combination with inhibition of miRNA such as miR-155 which promotes cancerogenesis, or mimicking miRNA whose under expression helps HCV replication, and mimicking downregulated miRNA with tumor suppressive function in HCC.

3. Nomenclature

The Family: Flaviviridae, Genus: Hepacivirus, species: Hepacivirus C.

(Guidelines of the International Committee on Virus Taxonomy (ICTV) https://talk.ictvonline.org/)

4. Conclusions

Chronic HCV infection ultimately leading to HCC will remain a global health problem in the coming decades. Despite increasing knowledge regarding mechanisms of HCV-induced HCC, prevention of HCV-induced HCC is not yet fully established. So, it is important to define both viral- and host genetic and epigenetic patterns on the onset of infection and in early and advanced stages of inflammation and fibrosis in order to predict response to the antiviral therapy, and thus avoid potential complications of persistent HCV infection. Further, for screening a population and making a correct diagnosis about the presence of infection, it is necessary to use standardized commercial tests with universal values and analyze homogenous group of patients. Despite new DAAs therapy, this virus remains unbeatable. When the heterogeneity of the HCV virus, the host genetic and epigenetic variability, and the differences in the therapy outcomes are taken into account, the only right way to fight this disease is personalized therapy.

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Conflict of interest

The authors declare that they have no conflict of interest.

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References

[1] World Health Organization. Guidelines for the Screening, Care and Treatment of Persons with Chronic Hepatitis C Infection. 2016. Available from: http://apps.who.int/iris/bitstream/10665/205035/1/9789241549615_eng.pdf (Accessed March 6, 2018)

[2] Poynard T, Yuen M-F, Ratziu V, Lai CL. Viral hepatitis C. Lancet. 2003;**362**:2095-2100

[3] Perz JF, Alter MJ. The coming wave of HCV-related liver disease: Dilemmas and challenges. Journal of Hepatology. 2006;**44**(3):441. DOI: 10.1016/j.jhep.2005.12.007

[4] Vo KP, Vutien P, Akiyama MJ, Vu VD, Ha NB, Piotrowski JI, et al. Poor sustained virological response in a Multicenter real-life cohort of chronic hepatitis C patients treated with Pegylated interferon and ribavirin plus telaprevir or boceprevir. Digestive Diseases and Sciences. 2015;**60**:1045-1051. DOI: 10.1007/s10620-015-3621-0

[5] Bukh J. The history of hepatitis C virus (HCV): Basic research reveals unique features in phylogeny, evolution and the viral life cycle with new perspectives for epidemic control. Journal of Hepatology. 2016;**65**:S2-S21. DOI: 10.1016/j.jhep.2016.07.035

[6] Pawlotsky J. New hepatitis C therapies: The toolbox, strategies, and challenges. Gastroenterology. 2014;**146**:1176-1192. DOI: 10.1053/j.gastro.2014.03.003

[7] Pawlotsky J-M. Hepatitis C virus resistance to direct-acting antiviral drugs in interferon-free regimens. Gastroenterology. 2016;**151**:70-86. DOI: 10.1053/j.gastro.2016.04.003

[8] Cavalcante LN, Lyra AC. Predictive factors associated with hepatitis C antiviral therapy response. World Journal of Hepatology. 2015;**7**:1617. DOI: 10.4254/wjh.v7.i12.1617

[9] Promrat K, McDermott DH, Gonzalez CM, Kleiner DE, Koziol DE, Lessie M, et al. Associations of chemokine system polymorphisms with clinical outcomes and treatment responses of chronic hepatitis C. Gastroenterology. 2003;**124**:352-360. DOI: 10.1053/gast.2003.50061

[10] Asselah T, Bièche I, Paradis V, Bedossa P, Vidaud M, Marcellin P. Genetics, genomics, and proteomics: Implications for the diagnosis and the treatment of chronic hepatitis C. Seminars in Liver Disease. 2007;**27**:013-027. DOI: 10.1055/s-2006-960168

[11] Saludes V, Bracho MA, Valero O, Ardèvol M, Planas R, González-Candelas F, et al. Baseline prediction of combination therapy outcome in hepatitis C virus 1b infected

patients by discriminant analysis using viral and host factors. PLoS One. 2010;**5**:e14132. DOI: 10.1371/journal.pone.0014132

[12] Jovanovic-Cupic S, Glisic S, Stanojevic M, Nozic D, Petrovic N, Mandusic V, et al. The influence of host factors and sequence variability of the p7 region on the response to pegylated interferon/ribavirin therapy for chronic hepatitis C genotype 1b in patients from Serbia. Archives of Virology. 2016;**161**:1189-1198. DOI: 10.1007/s00705-016-2777-z

[13] Jovanović-Ćupić S, Glisić S, Stanojević M, Vasiljević N, Bojić-Milinović T, Božović A, et al. Response factors to pegylated interferon-alfa/ribavirin treatment in chronic hepatitis c patients genotype 1b. Archives of Biological Sciences. 2014;**66**. DOI: 10.2298/ABS1401193J

[14] Antonucci G, Longo MA, Angeletti C, Vairo F, Oliva A, Comandini UV, et al. The effect of age on response to therapy with peginterferon ? Plus ribavirin in a cohort of patients with chronic HCV hepatitis including subjects older than 65 yr. The American Journal of Gastroenterology. 2007;**102**:1383-1391. DOI: 10.1111/j.1572-0241.2007.01201.x

[15] Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: A randomised trial. Lancet (London, England). 2001;**358**:958-965

[16] Fried MW, Hadziyannis SJ, Shiffman ML, Messinger D, Zeuzem S. Rapid virological response is the most important predictor of sustained virological response across genotypes in patients with chronic hepatitis C virus infection. Journal of Hepatology. 2011;**55**:69-75. DOI: 10.1016/j.jhep.2010.10.032

[17] Davis GL. Monitoring of viral levels during therapy of hepatitis C. Hepatology. 2002; **36**:S145-S151. DOI: 10.1002/hep.1840360719

[18] Poynard T, McHutchison J, Manns M, Trepo C, Lindsay K, Goodman Z, et al. Impact of pegylated interferon alfa-2b and ribavirin on liver fibrosis in patients with chronic hepatitis C. Gastroenterology. 2002;**122**:1303-1313

[19] Manns MP, Markova AA, Serrano BC, Cornberg M. Phase III results of Boceprevir in treatment naïve patients with chronic hepatitis C genotype 1. Liver International. 2012;**32**:27-31. DOI: 10.1111/j.1478-3231.2011.02725.x

[20] Parekh PJ, Shiffman ML. The role of interferon in the new era of hepatitis C treatments. Expert Review of Gastroenterology & Hepatology. 2014;**8**:649-656. DOI: 10.1586/17474124.2014.910453

[21] Benítez-Gutiérrez L, Barreiro P, Labarga P, de Mendoza C, Fernandez-Montero JV, Arias A, et al. Prevention and management of treatment failure to new oral hepatitis C drugs. Expert Opinion on Pharmacotherapy. 2016;**17**:1215-1223. DOI: 10.1080/14656566.2016.1182156

[22] Arias A, Aguilera A, Soriano V, Benítez-Gutiérrez L, Lledó G, Navarro D, et al. Rate and predictors of treatment failure to all-oral HCV regimens outside clinical trials. Antiviral Therapy. 2017;**22**:307-312. DOI: 10.3851/IMP3061

[23] Finkelmeier F, Dultz G, Peiffer K-H, Kronenberger B, Krauss F, Zeuzem S, et al. Risk of de novo hepatocellular carcinoma after HCV treatment with direct-acting antivirals. Liver Cancer. 2018. DOI: 10.1159/000486812

[24] Jovanović-Ćupić S, Kokanov N, Petrović N, Krajnović M, Kožik B, Kojić M SG. The influence of baseline viral and host factors on therapy response in patients with chronic hepatitis C genotype 1b from Serbia. First Congr. Mol. Biol. Serbia with Int. Particip., Belgrade, Serbia: n.d. p. 135

[25] Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, et al. Genome-wide association of IL28B with response to pegylated interferon-α and ribavirin therapy for chronic hepatitis C. Nature Genetics. 2009;**41**:1105-1109. DOI: 10.1038/ng.449

[26] Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'hUigin C, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature. 2009;**461**:798-801. DOI: 10.1038/nature08463

[27] Prokunina-Olsson L, Muchmore B, Tang W, Pfeiffer RM, Park H, Dickensheets H, et al. A variant upstream of IFNL3 (IL28B) creating a new interferon gene IFNL4 is associated with impaired clearance of hepatitis C virus. Nature Genetics. 2013;**45**:164-171. DOI: 10.1038/ng.2521

[28] Terczyńska-Dyla E, Bibert S, Duong FHT, Krol I, Jørgensen S, Collinet E, et al. Reduced IFNλ4 activity is associated with improved HCV clearance and reduced expression of interferon-stimulated genes. Nature Communications. 2014;**5**:5699. DOI: 10.1038/ncomms6699

[29] Miki D, Ochi H, Takahashi A, Hayes CN, Urabe Y, Abe H, et al. HLA-DQB1*03 confers susceptibility to chronic hepatitis C in Japanese: A genome-wide association study. PLoS One. 2013;**8**:e84226. DOI: 10.1371/journal.pone.0084226

[30] Duggal P, Thio CL, Wojcik GL, Goedert JJ, Mangia A, Latanich R, et al. Genome-wide association study of spontaneous resolution of hepatitis C virus infection: Data from multiple cohorts. Annals of Internal Medicine. 2013;**158**:235. DOI: 10.7326/0003-4819-158-4-201302190-00003

[31] Kuniholm MH, Kovacs A, Gao X, Xue X, Marti D, Thio CL, et al. Specific human leukocyte antigen class I and II alleles associated with hepatitis C virus viremia. Hepatology. 2010;**51**:1514-1522. DOI: 10.1002/hep.23515

[32] Singh R, Kaul R, Kaul A, Khan K. A comparative review of HLA associations with hepatitis B and C viral infections across global populations. World Journal of Gastroenterology. 2007;**13**:1770-1787. DOI: 10.3748/WJG.V13.I12.1770

[33] Knapp S, Warshow U, Hegazy D, Brackenbury L, Guha IN, Fowell A, et al. Consistent beneficial effects of killer cell immunoglobulin-like receptor 2DL3 and group 1 human leukocyte antigen-C following exposure to hepatitis C virus. Hepatology. 2010;**51**:1168-1175. DOI: 10.1002/hep.23477

[34] Khakoo SI, Thio CL, Martin MP, Brooks CR, Gao X, Astemborski J, et al. HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. Science. 2004;**305**:872-874. DOI: 10.1126/science.1097670

[35] Shaker O, El-Shehaby A, Fayez S, Zahra A, Marzouk S, Raziky M El. Osteopontin gene polymorphisms as predictors for the efficacy of interferon therapy in chronic hepatitis C Egyptian patients with genotype 4. Cell Biochemistry and Function. 2013:n/a-n/a. DOI:10.1002/cbf.2954

[36] Angelo ALD, Cavalcante LN, Abe-Sandes K, Machado TB, Lemaire DC, Malta F, et al. Myxovirus resistance, osteopontin and suppressor of cytokine signaling 3 polymorphisms predict hepatitis C virus therapy response in an admixed patient population: Comparison with IL28B. Clinics (São Paulo, Brazil). 2013;**68**:1325-1332. DOI: 10.6061/clinics/2013(10)06

[37] Eslam M, Hashem AM, Leung R, Romero-Gomez M, Berg T, Dore GJ, et al. Interferon-λ rs12979860 genotype and liver fibrosis in viral and non-viral chronic liver disease. Nature Communications. 2015;**6**:6422. DOI: 10.1038/ncomms7422

[38] Patin E, Kutalik Z, Guergnon J, Bibert S, Nalpas B, Jouanguy E, et al. Genome-wide association study identifies variants associated with progression of liver fibrosis from HCV infection. Gastroenterology. 2012;**143**:1244-1252.e12. DOI: 10.1053/j.gastro.2012.07.097

[39] Urabe Y, Ochi H, Kato N, Kumar V, Takahashi A, Muroyama R, et al. A genome-wide association study of HCV-induced liver cirrhosis in the Japanese population identifies novel susceptibility loci at the MHC region. Journal of Hepatology. 2013;**58**:875-882. DOI: 10.1016/j.jhep.2012.12.024

[40] Kumar V, Kato N, Urabe Y, Takahashi A, Muroyama R, Hosono N, et al. Genome-wide association study identifies a susceptibility locus for HCV-induced hepatocellular carcinoma. Nature Genetics. 2011;**43**:455-458. DOI: 10.1038/ng.809

[41] Heim MH, Bochud P-Y, George J. Host - hepatitis C viral interactions: The role of genetics. Journal of Hepatology. 2016;**65**:S22-S32. DOI: 10.1016/j.jhep.2016.07.037

[42] Huang H, Shiffman ML, Friedman S, Venkatesh R, Bzowej N, Abar OT, et al. A 7 gene signature identifies the risk of developing cirrhosis in patients with chronic hepatitis C. Hepatology. 2007;**46**:297-306. DOI: 10.1002/hep.21695

[43] Eslam M, Hashem AM, Romero-Gomez M, Berg T, Dore GJ, Mangia A, et al. FibroGENE: A gene-based model for staging liver fibrosis. Journal of Hepatology. 2016;**64**:390-398. DOI: 10.1016/j.jhep.2015.11.008

[44] Totoki Y, Tatsuno K, Covington KR, Ueda H, Creighton CJ, Kato M, et al. Trans-ancestry mutational landscape of hepatocellular carcinoma genomes. Nature Genetics. 2014; **46**:1267-1273. DOI: 10.1038/ng.3126

[45] Schulze K, Imbeaud S, Letouzé E, Alexandrov LB, Calderaro J, Rebouissou S, et al. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. Nature Genetics. 2015;**47**:505-511. DOI: 10.1038/ng.3252

[46] Ally A, Balasundaram M, Carlsen R, Chuah E, Clarke A, Dhalla N, et al. Comprehensive and integrative genomic characterization of hepatocellular carcinoma. Cell. 2017;**169**: 1327-1341.e23. DOI: 10.1016/j.cell.2017.05.046

[47] Chibon F. Cancer gene expression signatures—The rise and fall? European Journal of Cancer. 2013;**49**:2000-2009. DOI: 10.1016/j.ejca.2013.02.021

[48] Llovet JM, Chen Y, Wurmbach E, Roayaie S, Fiel MI, Schwartz M, et al. A molecular signature to discriminate dysplastic nodules from early hepatocellular carcinoma in HCV cirrhosis. Gastroenterology. 2006;**131**:1758-1767. DOI: 10.1053/j.gastro.2006.09.014

[49] Wu S-C, Chang SC, Wu H-Y, Liao P-J, Chang M-F. Hepatitis C virus NS5A protein downregulates the expression of spindle gene Aspm through PKR-p38 signaling pathway. The Journal of Biological Chemistry. 2008;**283**:29396-29404. DOI: 10.1074/jbc.M802821200

[50] Machida K, Liu J-C, McNamara G, Levine A, Duan L, Lai MMC. Hepatitis C virus causes uncoupling of mitotic checkpoint and chromosomal polyploidy through the Rb pathway. Journal of Virology. 2009;**83**:12590-12600. DOI: 10.1128/JVI.02643-08

[51] Lai C-K, Jeng K-S, Machida K, Cheng Y-S, Lai MMC. Hepatitis C virus NS3/4A protein interacts with ATM, impairs DNA repair and enhances sensitivity to ionizing radiation. Virology. 2008;**370**:295-309. DOI: 10.1016/J.VIROL.2007.08.037

[52] Martell M, Esteban JI, Quer J, Genescà J, Weiner A, Esteban R, et al. Hepatitis C virus (HCV) circulates as a population of different but closely related genomes: Quasispecies nature of HCV genome distribution. Journal of Virology. 1992;**66**:3225-3229

[53] Figlerowicz M, Alejska M, Kurzyńska-Kokorniak A, Figlerowicz M. Genetic variability: The key problem in the prevention and therapy of RNA-based virus infections. Medicinal Research Reviews. 2003;**23**:488-518. DOI: 10.1002/med.10045

[54] Muñoz de Rueda P, Fuentes Rodríguez JM, Quiles Pérez R, Gila Medina A, Martín Álvarez AB, Casado Ruíz J, et al. Hepatitis C virus NS5A region mutation in chronic hepatitis C genotype 1 patients who are non-responders to two or more treatments and its relationship with response to a new treatment. World Journal of Gastroenterology. 2017;**23**:4538. DOI: 10.3748/wjg.v23.i25.4538

[55] Munoz de Rueda P, Casado J, Paton R, Quintero D, Palacios A, Gila A, et al. Mutations in E2-PePHD, NS5A-PKRBD, NS5A-ISDR, and NS5A-V3 of hepatitis C virus genotype 1 and their relationships to pegylated interferon-ribavirin treatment responses. Journal of Virology. 2008;**82**:6644-6653. DOI: 10.1128/JVI.02231-07

[56] Saito I, Miyamura T, Ohbayashi A, Harada H, Katayama T, Kikuchi S, et al. Hepatitis C virus infection is associated with the development of hepatocellular carcinoma. Proceedings of the National Academy of Sciences of the United States of America. 1990;**87**:6547-6549

[57] Pawlotsky J-M. Pathophysiology of hepatitis C virus infection and related liver disease. n.d. DOI:10.1016/j.tim.2003.12.005

[58] NIH Consensus Statement on Management of Hepatitis C: 2002. NIH Consens State Sci Statements. n.d.;**19**:1-46

[59] McGivern DR, Lemon SM. Virus-specific mechanisms of carcinogenesis in hepatitis C virus associated liver cancer. Oncogene. 2011;**30**:1969-1983. DOI: 10.1038/onc.2010.594

[60] International Agency for Research on Cancer WHO. Liver Cancer Estimated Incidence, Mortality and Prevalence Worldwide in 2012. Globocan 2012 Estim Cancer Incid Mortal Preval Worldw 2012. http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx (Accessed March 6, 2018)

[61] Nagai H, Sumino Y. Therapeutic strategy of advanced hepatocellular carcinoma by using combined intra-arterial chemotherapy. Recent Patents on Anti-Cancer Drug Discovery. 2008;**3**:220-226

[62] Yang JD, Roberts LR. Hepatocellular carcinoma: A global view. Nature Reviews. Gastroenterology & Hepatology. 2010;**7**:448-458. DOI: 10.1038/nrgastro.2010.100

[63] Yoshida H, Tateishi R, Arakawa Y, Sata M, Fujiyama S, Nishiguchi S, et al. Benefit of interferon therapy in hepatocellular carcinoma prevention for individual patients with chronic hepatitis C. Gut. 2004;**53**:425-430. DOI: 10.1136/GUT.2003.030353

[64] Shiratori Y, Ito Y, Yokosuka O, Imazeki F, Nakata R, Tanaka N, et al. Antiviral therapy for cirrhotic hepatitis C: Association with reduced hepatocellular carcinoma development and improved survival. Annals of Internal Medicine. 2005;**142**:105-114

[65] D'Ambrosio R, Colombo M. Should surveillance for liver cancer be modified in hepatitis C patients after treatment-related cirrhosis regression? Liver International. 2016;**36**:783-790. DOI: 10.1111/liv.13106

[66] de Oliveria Andrade LJ, D'Oliveira A, Melo RC, De Souza EC, Costa Silva CA, Paraná R. Association between hepatitis C and hepatocellular carcinoma. Journal of Global Infectious Diseases. 2009;**1**:33-37. DOI: 10.4103/0974-777X.52979

[67] Vescovo T, Refolo G, Vitagliano G, Fimia GM, Piacentini M. Molecular mechanisms of hepatitis C virus-induced hepatocellular carcinoma. Clinical Microbiology and Infection. 2016;**22**:853-861. DOI: 10.1016/j.cmi.2016.07.019

[68] Bartosch B, Thimme R, Blum HE, Zoulim F. Hepatitis C virus-induced hepatocarcinogenesis. Journal of Hepatology. 2009;**51**:810-820. DOI: 10.1016/j.jhep.2009.05.008

[69] Nakamoto Y, Guidotti LG, Kuhlen CV, Fowler P, Chisari FV. Immune pathogenesis of hepatocellular carcinoma. The Journal of Experimental Medicine. 1998;**188**:341-350

[70] Mitochondrial injury, oxidative stress, and antioxidant gene expression are induced by hepatitis C virus core protein. Gastroenterology. 2002;**122**:366-375. DOI: 10.1053/GAST.2002.30983

[71] Helmut Bartsch JN. Oxidative stress and lipid peroxidation-derived DNA-lesions in inflammation driven carcinogenesis. Cancer Detection and Prevention. 2004;**28**:385-391. DOI: 10.1016/J.CDP.2004.07.004

[72] Gurtsevitch VE. Human oncogenic viruses: Hepatitis B and hepatitis C viruses and their role in hepatocarcinogenesis. The Biochemist. 2008;**73**:504-513. DOI: 10.1134/S0006297908050039

[73] Hoshida Y, Fuchs BC, Bardeesy N, Baumert TF, Chung RT. Pathogenesis and prevention of hepatitis C virus-induced hepatocellular carcinoma. Journal of Hepatology. 2014;**61**:S79-S90. DOI: 10.1016/j.jhep.2014.07.010

[74] Koike K. Hepatitis C virus contributes to hepatocarcinogenesis by modulating metabolic and intracellular signaling pathways. Journal of Gastroenterology and Hepatology. 2007;**22**:S108-S111. DOI: 10.1111/j.1440-1746.2006.04669.x

[75] Mesri EA, Feitelson MA, Munger K. Human viral oncogenesis: A cancer hallmarks analysis. Cell Host & Microbe. 2014;**15**:266-282. DOI: 10.1016/j.chom.2014.02.011

[76] Sakamuro D, Furukawa T, Takegami T. Hepatitis C virus nonstructural protein NS3 transforms NIH 3T3 cells. Journal of Virology. 1995;**69**:3893-3896

[77] Ray RB, Lagging LM, Meyer K, Ray R. Hepatitis C virus core protein cooperates with ras and transforms primary rat embryo fibroblasts to tumorigenic phenotype. Journal of Virology. 1996;**70**:4438-4443

[78] Ghosh AK, Majumder M, Steele R, Meyer K, Ray R, Ray RB. Hepatitis C virus NS5A protein protects against TNF-α mediated apoptotic cell death. Virus Research. 2000;**67**:173-178. DOI: 10.1016/S0168-1702(00)00141-6

[79] Ray RB, Meyer K, Ray R. Hepatitis C virus Core protein promotes immortalization of primary human hepatocytes. Virology. 2000;**271**:197-204. DOI: 10.1006/VIRO.2000.0295

[80] Park JS, Yang JM, Min MK. Hepatitis C virus nonstructural protein NS4B transforms NIH3T3 cells in cooperation with the ha-ras oncogene. Biochemical and Biophysical Research Communications. 2000;**267**:581-587. DOI: 10.1006/bbrc.1999.1999

[81] Egger G, Liang G, Aparicio A, Jones PA. Epigenetics in human disease and prospects for epigenetic therapy. Nature. 2004;**429**:457-463. DOI: 10.1038/nature02625

[82] Robertson KD. DNA methylation and human disease. Nature Reviews. Genetics. 2005; **6**:597-610. DOI: 10.1038/nrg1655

[83] Kaneto H, Sasaki S, Yamamoto H, Itoh F, Toyota M, Suzuki H, et al. Detection of hypermethylation of the p16 INK4A gene promoter in chronic hepatitis and cirrhosis associated with hepatitis B or C virus n.d

[84] Roncalli M, Bianchi P, Bruni B, Laghi L, Destro A, Di Gioia S, et al. Methylation framework of cell cycle gene inhibitors in cirrhosis and associated hepatocellular carcinoma. Hepatology. 2002;**36**:427-432. DOI: 10.1053/jhep.2002.34852

[85] Feinberg AP. Epigenetics at the epicenter of modern medicine. Journal of the American Medical Association. 2008;**299**:1345. DOI: 10.1001/jama.299.11.1345

[86] Feitelson M. Parallel epigenetic and genetic changes in the pathogenesis of hepatitis virus-associated hepatocellular carcinoma. Cancer Letters. 2006;**239**:10-20. DOI: 10.1016/j.canlet.2005.07.009

[87] Nishida N, Nagasaka T, Nishimura T, Ikai I, Boland CR, Goel A. Aberrant methylation of multiple tumor suppressor genes in aging liver, chronic hepatitis, and hepatocellular carcinoma. Hepatology. 2008;**47**:908-918. DOI: 10.1002/hep.22110

[88] Zekri AE-RN, Nassar AA-M, El-Din El-Rouby MN, Shousha HI, Barakat AB, El-Desouky ED, et al. Disease progression from chronic hepatitis C to cirrhosis and hepatocellular carcinoma is associated with increasing DNA promoter methylation. Asian Pacific Journal of Cancer Prevention. 2014;**14**:6721-6726

[89] Zekri A-RN, Bahnasy AA, Shoeab FEM, Mohamed WS, El-Dahshan DH, Ali FT, et al. Methylation of multiple genes in hepatitis C virus associated hepatocellular carcinoma. Journal of Advanced Research. 2014;**5**:27-40. DOI: 10.1016/j.jare.2012.11.002

[90] N Zekri A-R, Raafat AM, Elmasry S, Bahnassy AA, Saad Y, Dabaon HA, et al. Promotor methylation: Does it affect response to therapy in chronic hepatitis C (G4) or fibrosis? Annals of Hepatology. n.d.;**13**:518-524

[91] Arora P, Kim E-O, Jung JK, Jang KL. Hepatitis C virus core protein downregulates E-cadherin expression via activation of DNA methyltransferase 1 and 3b. Cancer Letters. 2008;**261**:244-252. DOI: 10.1016/j.canlet.2007.11.033

[92] Park S-H, Lim JS, Lim S-Y, Tiwari I, Jang KL. Hepatitis C virus Core protein stimulates cell growth by down-regulating p16 expression via DNA methylation. Cancer Letters. 2011;**310**:61-68. DOI: 10.1016/j.canlet.2011.06.012

[93] Huang J-Z, Xia S-S, Ye Q-F, Jiang H-Y, Chen Z-H. Effects of p16 gene on biological behaviours in hepatocellular carcinoma cells. World Journal of Gastroenterology. 2003;**9**:84-88

[94] Park J, Jang KL. Hepatitis C virus represses E-cadherin expression via DNA methylation to induce epithelial to mesenchymal transition in human hepatocytes. Biochemical and Biophysical Research Communications. 2014;**446**:561-567. DOI: 10.1016/j.bbrc.2014.03.009

[95] Lee S, Lee HJ, Kim J-H, Lee H-S, Jang JJ, Kang GH. Aberrant CpG island hypermethylation along multistep hepatocarcinogenesis. The American Journal of Pathology. 2003;**163**:1371-1378. DOI: 10.1016/S0002-9440(10)63495-5

[96] Feng Q, Stern JE, Hawes SE, Lu H, Jiang M, Kiviat NB. DNA methylation changes in normal liver tissues and hepatocellular carcinoma with different viral infection. Experimental and Molecular Pathology. 2010;**88**:287-292. DOI: 10.1016/j.yexmp.2010.01.002

[97] Chan KCA, Lai PBS, Mok TSK, Chan HLY, Ding C, Yeung SW, et al. Quantitative analysis of circulating methylated DNA as a biomarker for hepatocellular carcinoma. Clinical Chemistry. 2008;**54**:1528-1536. DOI: 10.1373/clinchem.2008.104653

[98] Zhang Y-J, Wu H-C, Shen J, Ahsan H, Tsai WY, Yang H-I, et al. Predicting hepatocellular carcinoma by detection of aberrant promoter methylation in serum DNA. Clinical Cancer Research. 2007;**13**:2378-2384. DOI: 10.1158/1078-0432.CCR-06-1900

[99] Iyer P, Zekri A-R, Hung C-W, Schiefelbein E, Ismail K, Hablas A, et al. Concordance of DNA methylation pattern in plasma and tumor DNA of Egyptian hepatocellular carcinoma patients. Experimental and Molecular Pathology. 2010;**88**:107-111. DOI: 10.1016/j.yexmp.2009.09.012

[100] Li Z, Zhang H, Yang J, Hao T, Li S. Promoter hypermethylation of DNA damage response genes in hepatocellular carcinoma. Cell Biology International. 2012;**36**:427-432. DOI: 10.1042/CBI20100851

[101] Hayashi T, Tamori A, Nishikawa M, Morikawa H, Enomoto M, Sakaguchi H, et al. Differences in molecular alterations of hepatocellular carcinoma between patients with a sustained virological response and those with hepatitis C virus infection. Liver International. 2009;**29**:126-132. DOI: 10.1111/j.1478-3231.2008.01772.x

[102] Djebali S, Davis CA, Merkel A, Dobin A, Lassmann T, Mortazavi A, et al. Landscape of transcription in human cells. Nature. 2012;**489**:101-108. DOI: 10.1038/nature11233

[103] Ling H, Vincent K, Pichler M, Fodde R, Berindan-Neagoe I, Slack FJ, et al. Junk DNA and the long non-coding RNA twist in cancer genetics. Oncogene. 2015;**34**:5003-5011. DOI: 10.1038/onc.2014.456

[104] Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, et al. The nuclear RNase III Drosha initiates microRNA processing. Nature. 2003;**425**:415-419. DOI: 10.1038/nature01957

[105] Koscianska E, Starega-Roslan J, Krzyzosiak WJ. The role of dicer protein partners in the processing of MicroRNA precursors. PLoS One. 2011;**6**:e28548. DOI: 10.1371/journal.pone.0028548

[106] Cheloufi S, Dos Santos CO, Chong MMW, Hannon GJ. A dicer-independent miRNA biogenesis pathway that requires ago catalysis. Nature. 2010;**465**:584-589. DOI: 10.1038/nature09092

[107] Monroig P del C, Chen L, Zhang S, Calin GA. Small molecule compounds targeting miRNAs for cancer therapy. Advanced Drug Delivery Reviews. 2015;**81**:104-116. DOI: 10.1016/j.addr.2014.09.002

[108] Jelen MM, Glavač D. Importance of MicroRNAs in hepatitis B and C diagnostics and treatment. In: Allam N, editor. Adv. Treat. Hepat. C B. Vol. 3. InTech; 2017. DOI: 10.5772/62815

[109] Petrovic N, Ergün S, Isenovic ER. Levels of MicroRNA heterogeneity in cancer biology. Molecular Diagnosis & Therapy. 2017;**21**:511-523. DOI: 10.1007/s40291-017-0285-9

[110] Varnholt H. The role of microRNAs in primary liver cancer. Annals of Hepatology. 2008;**7**:104-113

[111] Shrivastava S, Steele R, Ray R, Ray RB. MicroRNAs: Role in hepatitis C virus pathogenesis. Genes and Diseases. 2015;**2**:35-45. DOI: 10.1016/j.gendis.2015.01.001

[112] Motawi TMK, Sadik NAH, Shaker OG, Ghaleb MH. Elevated serum microRNA-122/222 levels are potential diagnostic biomarkers in Egyptian patients with chronic hepatitis C but not hepatic cancer. Tumor Biology. 2016;**37**:9865-9874. DOI: 10.1007/s13277-016-4884-6

[113] Ono C, Fukuhara T, Motooka D, Nakamura S, Okuzaki D, Yamamoto S, et al. Characterization of miR-122-independent propagation of HCV. PLoS Pathogens. 2017;**13**:e1006374. DOI: 10.1371/journal.ppat.1006374

[114] Pang PS, Pham EA, Elazar M, Patel SG, Eckart MR, Glenn JS. Structural map of a MicroRNA-122: Hepatitis C virus complex. Journal of Virology. 2012;**86**:1250-1254. DOI: 10.1128/JVI.06367-11

[115] Murakami Y, Aly HH, Tajima A, Inoue I, Shimotohno K. Regulation of the hepatitis C virus genome replication by miR-199a. Journal of Hepatology. 2009;**50**:453-460. DOI: 10.1016/j.jhep.2008.06.010

[116] Zhang Y, Wei W, Cheng N, Wang K, Li B, Jiang X, et al. Hepatitis C virus-induced up-regulation of microRNA-155 promotes hepatocarcinogenesis by activating Wnt signaling. Hepatology. 2012;**56**:1631-1640. DOI: 10.1002/hep.25849

[117] Petrovic N, Davidovic R, Jovanovic-Cupic S, Krajnovic M, Lukic S, Petrovic M, et al. Changes in miR-221/222 levels in invasive and in situ carcinomas of the breast: Differences in association with Estrogen receptor and TIMP3 expression levels. Molecular Diagnosis & Therapy. 2016;**20**:603-615. DOI: 10.1007/s40291-016-0230-3

[118] Braconi C, Valeri N, Gasparini P, Huang N, Taccioli C, Nuovo G, et al. Hepatitis C virus proteins modulate MicroRNA expression and Chemosensitivity in malignant hepatocytes. Clinical Cancer Research. 2010;**16**:957-966. DOI: 10.1158/1078-0432.CCR-09-2123

[119] Hsu S, Wang B, Kota J, Yu J, Costinean S, Kutay H, et al. Essential metabolic, anti-inflammatory, and anti-tumorigenic functions of miR-122 in liver. The Journal of Clinical Investigation. 2012;**122**:2871-2883. DOI: 10.1172/JCI63539

Section 4

Hepatitis C Treatment Strategies

Chapter 9

Safety, Tolerability, and Associated Side Effects of Direct-Acting Antivirals

Sidra Rehman

Additional information is available at the end of the chapter

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Abstract

Hepatitis C virus (HCV) infection is one of the major reasons for causing chronic hepatic disease worldwide. Treatment options for patients infected with chronic hepatitis C (CHC) have effectually ameliorated over the last few years. Now, various novel antiviral drugs have been licensed for its treatment. Introduction of direct-acting antivirals (DAAs) for HCV therapy represents a major advancement with regard to sustained virologic response (SVR) rates and associated adverse effect (AEs) profiling. Systematically, DAAs specifically impede different nonstructural proteins of HCV including NS3/4A protease, NS5A protein, and NS5B polymerase. In spite of those DAAs, therapy is confronting multiple challenges such as possible drug-drug interactions and severe side effects including liver failure. This chapter discusses the safety and tolerability of DAAs relevant to associated side effects emphasizing their clinical pharmacology. Considering the increased HCV prevalence rate and interpreting safety data of DAA regimens approved in the USA, Europe, Russia, Australia, and Japan, this chapter also presents the pre- and post-marketing safety data. Eventually, the important safety issues of drug–drug interactions (DDIs) have also been discussed in brief.

Keywords: hepatitis C virus, direct-acting antivirals, safety, tolerability, side effects

1. Introduction

Hepatitis C virus (HCV) infection, one of the major elements of liver disease worldwide [1], can cause both acute and chronic infections. Acute infection may follow an asymptomatic condition as well as self-limited hepatitis. Almost 15–45% of HCV-infected patients impulsively clear out the virus within 24 weeks of infection without getting any treatment. The remaining 55–85% of patients develop chronic infection; out of them 15–30% come across the risk of liver

cirrhosis within 20 years [2, 3]. Hepatitis C infection may cause liver histological changes, pervasive fibrosis, and cirrhosis with or without hepatic carcinoma. Furthermore, HCV infection may be associated with higher risk of cardiovascular disease [4, 5]. Advanced liver disease may lead to liver transplantation worldwide predisposing patients to a wide variety of clinical manifestations, thus progressing to liver-related mortality in due sequel [6]. Viral genome sequences are highly variable. About 11 different genotypes have been discovered so far having 30–50% nucleotide sequence variation; however, six genotypes are more prevalent in different regions. HCV genotype 1 is the most common among them representing 46.2% of all HCV cases followed by genotype 3 (30.1% of all HCV cases). The rest of the genotypes including genotype 2 (9.1% of all HCV cases), genotype 4 (8.3% of all HCV cases), genotype 5 (5.4% of all HCV cases), and genotype 6 (<1% of all HCV cases) are less commonly found globally. All these genotypes also vary for their pathogenicity, virulence, and progression rate to severe clinical manifestations [7]. For example, HCV infection with genotype 1b is related to more aggressive course of hepatic disease while comparing with other genotypes [8]. Liver cirrhotic patients and those having decompensated liver disease requiring liver transplantation have more commonly found genotype 1b infection than patients exhibiting chronically active HCV [9]. However, rapid fibrosis progression is associated with HCV genotype 3 [10]. Furthermore, each genotype responds differently to pharmacological treatment. Higher resistance to interferon therapy was shown in patients having genotypes 1 and 4 than with genotypes 2 and 3 [7].

Chronic HCV treatment is undergoing a continuous dynamic change. Since 1991 interferon-based therapy for chronic hepatitis C (CHC) patients was used as the standard of care (SOC), but therapy had unresponsive cure rate of no more than 6% along with serious side effects that led to the discontinuation of treatment subsequently [11]. In the start of millennium, pegylated interferon (pegIFN) with guanosine analogue, ribavirin (RBV), replaced the standard interferon as a safer and well-tolerated therapy regardless of HCV genotypes [12, 13]. Therapeutic progress was limited due to substantial adverse event (AE) profile and minimal response rates particularly in HCV genotype 1-infected patients [14]. Side effects include bone marrow depression with lower content of granulocytes, neutropenia, and flu-like symptoms. Neuropsychiatric side effects such as irritability, severe fatigue, and apathy are extensive problems for patients and their families if not treated properly on time [15]. Furthermore, autoimmune diseases like rheumatoid arthritis, psoriasis, vitiligo, lichen planus, dermatitis herpetiformis, type 1 diabetes mellitus, and sarcoidosis can be enrooted or intensified during pegIFN therapy [15]. Therefore, therapy is advised with vigilance to patients having autoimmune disease. Thyroid dysfunction and RBV-associated side effects are also linked with pegIFN therapy. The abovementioned side effects, prolonged duration, high cost, and lesser adherence to therapy lead to the discontinuation of treatment.

A new era of HCV therapy was heralded with the emergence and approval of oral direct acting antiviral (DAA) agents. In comparison to nonspecificity of IFN-based therapy, DAAs directly intend to block various proteins involved in HCV replication pathways. Recently, available DAAs inhibit nonstructural proteins including NS3/4A protease, NS5A protein, and NS5B polymerase alone or in combination with other antiviral agents involved in blocking different stages of HCV replication [16]. Four main classes of DAAs used in multiple combinations for HCV treatment are described in **Table 1** [17]. First-generation protease inhibitors, telaprevir® and boceprevir®, were

Classes of DAAs	Target proteins/enzymes	Dose/combination used/year of approval	Targeted genotypes	With or without cirrhosis	Cure rate/course of treatment	Cost of therapy ($)
NS3/NS4A protease inhibitors	NS3/NS4A inhibitors act to block viral protease that help virus in posttranslational processing and HCV replication	1. Glecaprevir (in combination with pibrentasvir) (2016) 2. Simeprevir (2014)	1. All six major genotypes 2. Genotypes 1a/b	1. Without cirrhosis 2. Without cirrhosis	1. 90–100%/8–12 weeks 2. 80–90%/12 weeks	$26,400/ treatment course $66,360/ treatment course
Nucleoside and nucleotide NS5B polymerase inhibitors	These inhibitors directly act to block viral replication by attaching to viral RNA polymerase enzyme	1. Epclusa (sofosbuvir and velpatasvir) (2016) 2. Sofosbuvir (2014)	1. All six major genotypes 2.Genotypes 1–4	1. Both 2. Without cirrhosis	1. 100% (some groups); 94% for decompensated cirrhosis/12 weeks 2. 30 –97%/12–24 weeks	$74,760 $84,000 in the USA
NS5A inhibitors	Block directly NS5A protein which is required for HCV reproduction	1. Zepatier (elbasvir/ grazoprevir) (2016) 2. Daclatasvir (2015) 3. Ombitasvir with paritaprevir and ritonavir (2015) 4. Ledipasvir with sofosbuvir (2014)	1. Genotypes 1 and 4 2. Genotype 3 3. Genotypes 1 and 4 4. Genotype 1	1. Both 2. Both 3. Without cirrhosis 4. Both	1. 94–97% (genotype 1); 97–100% (genotype 4)/12–16 weeks 2. 98% (treatment-naïve) and 58% (treatment experienced patients with cirrhosis)/12 weeks 3. 100% (treatment-naïve)/12 weeks 4. 93–99%/12–24 weeks	$54,600 for 12 weeks $63,000 in the USA $76,653 $94,500; 12 weeks
Non-nucleoside NS5B polymerase inhibitors	They work to block HCV from reproduction by inserting themselves into virus so that other viral pieces cannot attach to it	1. Dasabuvir (in combination with paritaprevir and ombitasvir) (2014)	1. Genotype 1a/1b	1. Both, for compensated cirrhosis (used with RBV)	1. >90%/12–24 weeks	$83,319 in the USA for 12 weeks

Table 1. List of direct acting antivirals (DAAs).

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approved in 2011 by the European Medical Academy (EMA) and Food and Drug Administration (FDA) for the treatment of CHC patients of genotype 1, but due to severe cutaneous AEs including diffuse rash or localized rash with pruritus and skin peeling, therapy was discontinued in 2012 [18, 19]. In 2014, the EMA approved four new polymerase/protease inhibitors (simeprevir®, sofosbuvir®, ledipasvir®, and daclatasvir®) having variable pharmacodynamic characteristics such as simeprevir® which inhibits NS3/4A protease, sofosbuvir® which blocks NS5B polymerase, and ledipasvir® and daclatasvir® which are HCV NS5A inhibitors. The Japanese Ministry of Health, Labor and Welfare approved an NS3/4A protease inhibitor, asunaprevir®, in 2014 for the treatment of genotypes 1 and 4. Combination of asunaprevir® with daclatasvir® is the first oral interferon and RBV-free treatment for CHC patients having genotype 1 infection. This combinatorial treatment is also approved in Australia and Russia. In the following year, the EMA and FDA approved a new combination of drugs ombitasvir®/paritaprevir®/ritonavir®. Among these, ritonavir® is not active against HCV infection; rather, it is a cytochrome P450 3A (CYP3A) inhibitor which increases systemic exposure of paritaprevir® (a CYP3A substrate). Ombitasvir® is an HCV NS5A inhibitor. In the same year, dasabuvir®, NS5B polymerase inhibitor, was also approved by the EMA. Combination of all these drugs with or without RBV can be used for the treatment of HCV genotypes 1a, 1b, and 4 [20]. In January 2016, the FDA approved zepatier® (grazoprevir®/elbasvir®) for marketing. In May 2016, the EMA also recommended the granting of marketing authorizations in the EU for zepatier® treatment [20]. In the same year, epclusa® (combination of sofosbuvir® and velpatasvir®) was approved for the treatment of all six major genotypes. Recently, in July 2017, Vosevi® (combination of sofosbuvir®/velpatasvir®/voxilaprevir®) was approved by the FDA in which each drug acts through a different mechanism [21]. Vosevi® includes combination of NS5B polymerase, NS3/NS4A, and NS5A inhibitors.

2. General safety and tolerability aspects of DAAs

The basic purposes of HCV pharmacological therapy are to eliminate viral infection and to prevent it from causing cirrhosis and associated complications. Alleviative effects of viral pharmacological therapies are estimated through the sustained virological response (SVR) which is defined as a viremia (undetectable HCV RNA in blood) 12 or 24 weeks after completion of anti-HCV therapy. In clinical trials, SVR is commonly used as primary efficacy end point and serves as the only factor linked with liver-associated events and all-cause mortality [22, 23].

2.1. Clinical efficacy and tolerability profile related to DAAs from premarketing studies

Fundamental characteristics of premarketing studies have been enlisted in **Table 2**. Antiviral drug's efficacy and tolerability profile of different DAA combinations are discussed below.

2.1.1. Clinical tolerability analysis of sofosbuvir® treatment in different combinations

Clinical efficacy results of sofosbuvir pivotal studies including NEUTRINO, FISSION, POSITRON, and FUSION trials depicted that the most usual AEs were nausea, headache, fatigue,

and insomnia. In these trials more than 1000 patients of all six major HCV genotypes were treated with sofosbuvir® and RBV or PegIFN and RBV. Mostly, severe AEs were observed in patients treated with PegIFN and RBV as compared to sofosbuvir® [24, 25]. According to safety results of NEUTRINO and FISSION trials, fever, depression, and influenza-like symptoms were more common in PegIFN-treated patients as compared to those who received sofosbuvir®. Lastly, POSITRON safety data illustrated that gastrointestinal disorder, administration site reactions,

Treatment/study group/ year	Population	Adverse event (AE) profile	Overall SVR_{12} rate	Treatment status
SOF/RBV (n = 256) vs. PegIFNα-2a/RBV (n = 243) (2013) [23]	499 genotypes 2/3 HCV patients	More patients treated with PegIFNα-2a/RBV had AEs. Common AEs in patients treated with SOF/RBV and PegIFNα-2a/RBV were fatigue (36 vs. 55%), headache (25 vs. 44%), nausea (18 vs. 29%), insomnia (12 vs. 29%), anemia (8 vs. 12%), influenza-like symptoms (3 vs. 16%), fever (3 vs.18%), and depression (5 vs. 14%)	67%	26 patients treated with PegIFNα-2a/RBV and three patients treated with SOF/RBV discontinued their treatment
SOF/RBV/PegIFNα-2a (2013) [23]	327 genotypes 1, 4, 5, or 6 HCV patients	The most common AEs included fatigue (59%), headache (36%), nausea (34%), insomnia (25%), and anemia (21%)	90%	8 patients
SOF/RBV (n = 207) vs. placebo/RBV (n = 71) (2013) [24]	278 genotype 2 or 3 HCV patients	The most common AEs by SOC in SOF/RBV and RBV/placebo groups were general disorders and administration site reactions (57 vs. 36.6%), gastrointestinal disorders (43.5 vs. 39.4%), nervous system disorders (35.3 vs. 29.6%), musculoskeletal and connective disorders (18.8 vs. 7%), blood and lymphatic system disorders (14%vs.1.4%), complications (9.2 vs. 5.6%),and metabolism and nutrition disorders (7.2 vs. 12.7%)	78%	4 patients who received SOF/RBV and three patients who received placebo (4%)
SOF/RBV for 12 weeks (n = 103) vs. SOF/RBV for 16 weeks (n = 98) (2013) [24]	201 genotype 2 or 3 HCV patients	The most common AEs by SOC in SOF/RBV 12 weeks and SOF/RBV 16 weeks groups were general disorders and administration site reactions (58.3 vs. 60.2%), gastrointestinal disorders (47.6 vs. 46.9%), infections and infestations (31.1 vs. 23.5%), nervous system disorders (36.9 vs. 42.9%), musculoskeletal and connective disorders (28.2 vs. 34.7%), psychiatric disorders (34 vs. 41.8%), and skin and subcutaneous tissue disorders (33 vs. 31.6%)	50%	1 patient in SOF/RBV 12 weeks group

Treatment/study group/ year	Population	Adverse event (AE) profile	Overall SVR_{12} rate	Treatment status
DCV + SOF ± RBV for 12 weeks (n = 103) vs. 24 weeks (n = 302) (2017) [25]	617 HIV-/HCV-coinfected patients with genotype 1, 3, and 4	Common AEs associated with DCV + SOF ± RBV were decompensated cirrhosis/multi-organ failure, respiratory disorder, hepatic carcinoma, lymphopenia, and renal insufficiency	Overall 92%	7 patients discontinued
LDV/SOF for 12 weeks (n = 109) vs. LDV/SOF/ RBV for 12 weeks (n = 111) vs. LDV/SOF for 24 weeks (n = 109) vs. LDV/SOF/ RBV for 24 weeks (n = 111) (2014) [26]	440 genotype 1 HCV patients (20% with cirrhosis)	Six percent of patients in LDV/ SOF for 24 weeks and 3% LDV/ SOF/RBV for 24 weeks had a serious of AEs (P = 0.36) More patients in RBV groups had fatigue, nausea, insomnia, arthralgia, cough, rash, irritability, dyspnea, and anemia. Grade 1 or 2 hyperbilirubinemia occurred in more patients who received LDV/SOF/RBV for 12 and 24 weeks compared to patients who received LDV/SOF for 12 and 24 weeks (32 and 41% vs. 1 and 7%, respectively)	>90%	No AEs leading to treatment discontinuation
LDV/SOF for 8 weeks (n = 215) vs. LDV/SOF/ RBV for 8 weeks (n = 216) vs. LDV/SOF for 12 weeks (n = 216) (2014) [27]	647 previously untreated patients with HCV genotype 1 infection	The most common AEs in LDV/ SOF for 8 weeks, LDV/SOF/RBV for 8 weeks, and LDV/SOF for 12 weeks groups were fatigue (21 vs. 35 vs. 23%), headache (14 vs. 25 vs. 15%), nausea (7 vs. 18 vs. 11%), and insomnia (5 vs. 12 vs. 7%) Fatigue, headache, nausea, insomnia, irritability, rash, pruritus, cough, and anemia were more common in patients treated with RBV Three patients in the group LDV/SOF/RBV for 8 weeks had grade 3 hyperbilirubinemia	93–95%	1 patient in LDV/ SOF/RBV for 8 weeks and two patients in LDV/ SOF for 12 weeks
DCV/SOF for 23 weeks (groups A and B; n = 31) vs. DCV/SOF for 24 weeks (groups C and D; n = 28) vs. DCV/SOF/RBV for 24 weeks (groups E and F; n = 29) DCV/SOF, with or without RBV, for 12 weeks (group G; n = 41 or group H; n = 41) or 24 weeks (41 patients who did not have a response to prior treatment with HCV protease inhibitors, (n = 21) or J (n = 20) (2014) [28]	211 genotypes 1–3 HCV patients	Common AEs occurred in groups A and B; C and D; E and F; and G, H, I, and J were fatigue (29 vs. 50 vs. 31 vs. 39 vs. 37 vs. 29 vs. 45%), headache (16 vs. 29 vs. 38 vs. 34 vs. 22 vs. 33 vs. 35%), and nausea (16 vs. 32 vs. 31 vs. 20 vs. 20 vs. 10%)	>90%	1 patient in groups C and D (DCV/ SOF) and one patient in groups E and F (DCV/SOF/ RBV) discontinued the treatment

Treatment/study group/ year	Population	Adverse event (AE) profile	Overall SVR_{12} rate	Treatment status
DCV 20 mg/PegIFN/RBV (n = 159) vs. DCV 60 mg/ PegIFN/RBV (n = 158) vs. placebo/PegIFN/RBV (n = 78) (2015) [29]	395 treatment-naïve patients with HCV genotype 1 or 4	A higher percentage of patients in placebo/PegIFN/RBV group had treatment failure compared to patients in DCV 20 mg or 60 mg groups (62.5 vs. 40.4 vs. 40.8%) Among the most common AEs in DCV 20, 60 mg, and placebo group, there were fatigue (55.3 vs. 54.4 vs. 59%), headache (42.8 vs. 43 vs. 46.2%), pruritus (35.2 vs. 39.9 vs. 33.3%), insomnia (30.8 vs. 33.5 vs. 38.5%), and rash (34 vs. 25.3 vs. 32.1%)	>90%	7 patients in DCV 20 mg group, seven patients in DCV 60 mg group, and eight patients in placebo group
DCV/SOF/RBV for 12 (n = 24) or 16 weeks (n = 26) (2016) [30]	50 treatment-naïve (n = 13) or treatment-experienced (n = 37) genotype 3 patients with advanced fibrosis (n = 14) or compensated cirrhosis (n = 36)	The most common AEs occurring in at least 10% of patients in DCV/SOF/RBV for 12 weeks or 16 weeks groups were insomnia (33.3 vs. 26.9%), fatigue (25 vs. 26.9%), headache (29.2 vs. 19.2%), irritability (20.8 vs. 7.7%), asthenia (8.3 vs. 19.2%), and diarrhea (4.2 vs.15.4%)	Overall 90%	No AEs leading to treatment discontinuation
SMV/PegIFN/RBV (n = 260) vs. placebo/ PegIFN/RBV (n = 133) (2014) [31]	393 genotype 1 HCV patients	Frequently associated AEs in SMV/PegIFN/RBV and placebo groups were fatigue (31.9 vs. 42.1%), headache (31.9 vs. 36.1%), and influenza-like symptoms (29.6 vs. 20.3%) 2 patients in SMV group had grades 2/3 photosensitivity events 6.2% of patients SMV/ PegIFN/RBV had grades 3/4 hyperbilirubinemia (the frequency in placebo group was 3.1%)	80–83%	0.4% of patients in SMV/PegIFN/RBV
SMV + SOF ± RBV for 12 weeks vs. 24 weeks (2014) [32]	167 genotype 1 chronic HCV patients	Most common AEs in pooled groups of patients treated with different simeprevir/sofosbuvir combinations included headache (20%), nausea (16%), and fatigue (31%). Grade 4 AEs were observed in one patient (2%) in each of groups 1 and 3, in three patients (10%) of group 2, while grades 3–4 AEs were scrutinized in less than 5% of the patients except the elevated level of blood amylase	92 and 94% for cohorts 1 and 2	4 patients (2%) had withdrawn from all study treatment due to AEs and three patients discontinued before week 12

Treatment/study group/ year	Population	Adverse event (AE) profile	Overall SVR_{12} rate	Treatment status
DCV 60 mg QD + ASV 200 mg BID × 24 weeks (group A1; n = 18) vs. DCV 60 mg QD + ASV 200 mg QD ×2 4 weeks (group A2; n = 20) vs. DCV 60 mg QD + ASV 200 mg BID + PegIFNα/RBV × 24 weeks (groupB1; n = 20) vs. DCV 60 mg QD + ASV 200 mg QD + PegIFNα/RBV × 24 weeks (group B2; n = 21) vs. DCV 60 mg QD + ASV 200 mg BID + RBV × 24 weeks (group B3; n = 22) (2014) [33]	101 genotype 1a and 1b HCV patients	Frequently associated AEs in all groups were headache (44 vs. 40 vs. 60 vs. 48 vs. 46%), diarrhea (28 vs. 30 vs. 45 vs. 33 vs. 23%), fatigue 28 vs. 10 vs. 40 vs. 24 vs. 32%), asthenia (17 vs. 20 vs. 30 vs. 57 vs. 32%), myalgia (22 vs. 5 vs. 10 vs. 38 vs. 9%), and insomnia (17 vs. 15 vs. 45 vs. 14 vs. 41%)	Group A1 (78%), group A2 (65%), group B1 (95%), and group B2 (95%)	1 patient in B3 group
DCV/ASV/PegIFN/RBV for 24 weeks (2015) [34]	398 genotype 1 or 4 chronic HCV patients	Frequently associated AEs were fatigue (41.5%), headache (31.2%), pruritus (26.1%), asthenia (24.1%), influenza-like illness (22.4%), and insomnia (22.4%)	Genotype 1 (93%) and genotype 4 (98%)	18 patients
PrOD with RBV (group A) (n = 437) vs. matching placebos (group B) (n = 158) (2014) [35]	595 previously untreated genotype 1 HCV patients	Common AEs in groups A and B ($P < 0.05$ for each comparison) were fatigue (34.7 vs. 28.5%), headache (33.0 vs. 26.6%), nausea (23.7 vs. 13.3%), pruritus (16.9 vs. 3.8%), insomnia (14.0 vs. 7.6%), diarrhea (13.7 vs. 7.0%), and asthenia (12.1 vs. 3.8%)	Genotype 1a (95%) and genotype 1b (98%)	3 Patients in group A and 1 patient in group B
PrOD with RBV (n = 297) vs. placebo (n = 97) during the 12-week double-blind period/2014 [36]	394 Previously treated with PegIFN/RBV who had a relapse, a partial response, or a null response in patients with HCV genotype 1 infection without cirrhosis	Common AEs in active and placebo groups were headache (36.4 vs. 35.1%; $P = 0.90$), fatigue (33.3 vs. 22.7%; $P = 0.06$), and nausea (20.2 vs. 17.5%). More patients in the active-regimen group had anemia ($P = 0.01$), and vomiting ($P = 0.006$); while AEs with a higher frequency in the placebo group were constipation ($P = 0.02$), erythema ($P = 0.05$), neck pain ($P = 0.05$), and neutropenia ($P = 0.01$).	96.3% overall	3 patients in the active regimen group
86 patients were treatment-naïve (44 received PrOD and 42 received PrOD/RBV) and 49 treatment-experienced patients received the RBV-containing therapy/2015 [37]	135 genotype 4 chronic HCV	Most common AEs were headache (29% of treatment-experienced patients vs. 33% of 42 treatment-naïve patients), asthenia (24 vs. 33%), fatigue (7 vs. 18%), insomnia (5 vs. 16%), and nausea (9 vs. 17%)	100% RBV-containing regimen and 90.9% RBV-free regimen	No AEs leading to treatment discontinuation

Treatment/study group/ year	Population	Adverse event (AE) profile	Overall SVR_{12} rate	Treatment status
PrOD with RBV (group 1) vs. PrOD (group 2)/ 2014 [38]	179 patients with HCV genotype 1b infection, without cirrhosis, previously treated with PegIFN/RBV	The most frequently reported AEs in groups 1 and 2 were fatigue (31.9 vs. 15.8%), headache (24.2 vs. 23.2%), and nausea (20.9 vs. 6.3%). Patients treated with RBV had more commonly insomnia, anemia, rash, and increased blood bilirubin levels	Group 1 97% and group 2 100%	1 patient in group 1 and 1 patient in group 2
GZP/EBR (immediate treatment group) (n = 111) vs. placebo (deferred treatment group) (n = 113)/2015 [39]	224 patients with HCV genotype 1 infection and chronic kidney disease	Common AEs occurred in patients in immediate treatment and deferred treatment groups were headache (17.1 vs. 16.8%), nausea (15.3 vs. 15.9%), fatigue (9.9 vs. 15%), insomnia (6.3 vs. 10.6%), dizziness (5.4 vs. 15.9%), and diarrhea (5.4 vs. 13.3%)	99%	5 patients in deferred treatment group
GZP/EBR/ 2015 [40]	218 treatment-naïve patients with chronic HCV genotype 1, 4, or 6 infection and HIV co-infection, with or without cirrhosis	The most frequent AEs were fatigue (13%), headache (12%), and nausea (9%).	96%	There were no AEs leading to treatment discontinuation

Table 2. Clinical efficacy and tolerability of direct acting antivirals (DAAs).

and nervous system disorders were mostly found in sofosbuvir®/RBV-treated patients as compared to the placebo group [24, 25]. Efficacy and safety of daclatasvir®/sofosbuvir® with or without RBV was assessed in more than 600 patients with advanced HCV disease. Population was mostly cirrhotic (72%, of whom 18% were decompensated), HCV treatment-experienced (82%), and infected with genotypes 1 (69%), 3 (12%), or 4 (19%). Most of them were treated for 24 weeks and 14% received RBV. Twelve weeks of SVR was 92% overall, 90% in cirrhotic patients, and 95% in non-cirrhotic patients. Twelve weeks of SVR (SVR12) remained constant among all major six genotypes and antiretroviral regimens. Among 617 patients with safety data, seven patients discontinued due to adverse events and ten died. About three out of seven reported discontinuation as AEs were consequently found fatal (decompensated cirrhosis/multi-organ failure, respiratory disorder, hepatic carcinoma) and for the remaining four were nonfatal (treatment-associated lymphopenia, renal insufficiency) [26]. Daclatasvir®/sofosbuvir® with or without RBV achieved high SVR12 and was well tolerated in this large real-world cohort of HIV-/HCV-coinfected patients with advanced liver disease. Conclusively, it is found that daclatasvir®/sofosbuvir® with or without RBV is well tolerated in real-world HIV-/HCV-coinfected cohort with advanced hepatic disease, and treatment is more suitable in this perspective [26].

Safety profile of sofosbuvir®/ledipasvir® combination with or without RBV was evaluated in pivotal study trials including ION-1, ION-2, and ION-3. In these clinical trials, 2000 HCV-infected

patients (cirrhotic and non-cirrhotic) with genotype 1 were treated with ledipasvir®/sofosbuvir® or ledipasvir®/sofosbuvir® plus RBV. Ledipasvir® was found to be associated with the occurrence of headache, insomnia, nausea, and asthenia. In data obtained from ION-2 and ION-3 trials, AEs such as cough, rash, grade 1 or 2 hyperbilirubinemia, arthralgia, anemia, irritability, and dyspnea were found frequently among patients treated with RBV [27, 28]. Furthermore, outcomes of treatment combinations like daclatasvir®/pegIFN/RBV vs. placebo, daclatasvir®/sofosbuvir®, and with or without RBV, were evaluated in ALLY-3+, AI444040, and AI444010 clinical studies, respectively, in more than 650 patients having genotype of 1, 2, 3, or 4. Like other DAAs symptoms like asthenia, headache, and nausea were more frequently observed [29–31]. AI444040 study illustrated discontinuation of therapy in two patients, one having stroke with history of hyperlipidemia, smoking, and myocardial infarction and the other having fibromyalgia exacerbation with history of fibromyalgia [29]. According to another efficacy study, in placebo group a large number of patients had grade 3–4 AEs when compared with patients who received daclatasvir® 20 or 60 mg (23.1 vs. 20.1 vs. 14.6%). In 3.8% of patients receiving 60 mg daclatasvir®, an increased alanine aminotransferase (ALT) level was observed vs. 1.3% of patients in placebo group, while patients receiving 20 mg daclatasvir® did not experience this side effect. However, both groups of drug dosage experienced the symptoms of influenza-like syndrome, nausea, and dry skin [30]. In ALLY-3+ phase III study trial, no AEs were observed in patients receiving daclatasvir®/sofosbuvir®, with RBV which led to discontinuation of therapy. The most common treatment-associated general side effects including irritability, insomnia, asthenia, fatigue, dyspnea, and diarrhea were observed in 10% of the patients. The high level of safety and clinical efficacy was demonstrated in patients having challenging viral 3a genotype who were administered with this combination for 12 or 16 weeks [31].

2.1.2. Efficacy and safety analysis of simeprevir® in different combinations

Simeprevir® in combination with PegIFNα and RBV was assessed in more than 1000 patients using QUEST-1, QUEST-2, and PROMISE clinical trials. AEs associated with simeprevir® included rash, increased bilirubin level, pruritus, and photosensitivity events as compared to the placebo group. No differences were found in PROMISE trial between simeprevir®/PegIFN/RBV and placebo groups for frequency of grades ¾ AEs [32]. Simeprevir® in combination with sofosbuvir® was approved by the FDA in 2014 for chronic patients of genotype 1. In COSMOS randomized study, patients were grouped in ratio of 2:1:2:1 who received simeprevir® (150 mg) and sofosbuvir® (400 mg) for 24 weeks with (group 1) or without (group 2) RBV and for 12 weeks with (group 3) or without (group 4) RBV, in two different cohorts: previously nonresponders having METAVIR[1] scores of F0–F2 and treatment-naïve patients and previously nonresponders with F3–F4 scores of METAVIR [33]. In cohorts 1 and 2, 92 and 94% of patients achieved SVR12, respectively. Most common AEs in pooled groups of patients treated with different simeprevir®/sofosbuvir® combinations included headache (20%), nausea (16%), and fatigue (31%). Grade 4 AEs were observed in one patient (2%) in each of groups 1 and 3 and in three patients (10%) of group 2, while grades 3–4 AEs were scrutinized in less than 5% of the patients except the elevated level of blood amylase. Serious

[1]Scoring system used to assess the extent of inflammation and fibrosis via histopathological evaluation in liver biopsy of HCV patients.

AEs were observed in four patients (2%); four patients (2%) had withdrawn from all study treatment due to AEs, and three patients discontinued before week 12 [33].

2.1.3. Tolerability analysis of asunaprevir® combinations

In a randomized phase 2a open-label study, safety and tolerability of asunaprevir®/daclatasvir® combination therapy was assessed in 100 patients of genotype 1a and 1b. Patients received five different regimens including daclatasvir® and asunaprevir® alone, at different dosages, or plus PegIFN/RBV. Efficacy data depicted that asthenia, headache, and diarrhea were more common and that serious hematological side effects were found in patients who received PegIFN and/or RBV. Correspondingly, flu-like illness, alopecia, and rash were observed in patients treated with PegIFN and/or RBV compared to those who treated with daclatasvir® and asunaprevir® [34]. During trials six severe AEs were analyzed: one patient got panic attack, one case of forearm fracture, one case of prostate cancer found in patients who received asunaprevir®/daclatasvir® combination, two over dosage cases, and one case of squamous cell carcinoma in patients treated with daclatasvir®/asunaprevir®/ PegIFN/ RBV [33]. In phase 3 study of HALLMARKQUAD trial, patients with genotype 1 (n = 354) or genotype 4 (n = 44) (partial or nonresponders to PegIFN/ RBV) were treated daily with daclatasvir®/asunaprevir® combination and weekly with PegIFN and/or RBV. In serious AEs (5.5% of patients), grade ¾ clinical manifestations included lymphopenia, thrombocytopenia, neutropenia, anemia, and elevated level of ALT/aspartate transaminase (AST) were found in the treated patients. Fatigue, pruritus, influenza-like symptoms, rash, asthenia, and insomnia were common AEs observed during study. Negligible difference was found in ALT and AST elevation among patients with or without cirrhosis [35]. Drug combination was well tolerated, and no additional safety concerns were identified in comparison to pegIFN/RBV regimens.

2.1.4. Efficacy study of ombitasvir®/paritaprevir®/ritonavir®

Safety analysis of ombitasvir®/paritaprevir®/ritonavir® in combination with dasabuvir® was analyzed among 1025 patients during clinical trials of SAPPHIRE-I and SAPPHIRE-II. Clinical efficacy data depicted that headache and fatigue were the most frequent AEs. In comparison to placebo group, patients treated with ombitasvir®/paritaprevir®/ritonavir®, dasabuvir®, and RBV were found to exhibit asthenia, diarrhea, pruritus, nausea, and insomnia as the most common AEs. On the other hand, placebo group patients were confronted to have erythema, neck pain, and constipation. Ventricular extra systoles, sinus tachycardia, acute respiratory failure, and acute transient stroke were reported as severe AEs in study treatment [36, 37]. In PEARL-I and PEARL-II trials, safety results in HCV-infected patients of genotypes 1 and 4 who were treated with combination of ombitasvir®/paritaprevir®/ritonavir®, with or without dasabuvir®, and RBV® illustrated that insomnia, nausea, asthenia, and headache were the most common AEs [38, 39].

2.1.5. Safety analysis of grazoprevir®/elbasvir® combination

According to C-SURFER safety study data, in which 244 patients of viral genotype 1 along with chronic kidney disease (CKD) were included, grazoprevir®/elbasvir® combination was

associated with AEs like nausea, headache, and fatigue. In two patients cardiac arrest and myocardial infarction were observed, while in placebo group, three patients were reported with serious cardiac events. Though the severities and frequencies of liver-associated events were comparable between groups, elevation in ALT and AST levels were more frequent in placebo group. In grazoprevir®/elbasvir®-treated group of patients, a low hemoglobin level (24.3 vs. 16.8%) was recorded [40]. In single-arm C-EDGE COINFECTION phase 3 study, safety of grazoprevir®/elbasvir® was assessed in patients (n = 218) of genotype 1, 4, or 6 coinfected with HIV. Related to the previous study, the most frequent AEs were nausea, fatigue, and headache [41].

2.2. Post-marketing safety reports of DAAs

Post-marketing survey studies scrutinizing the efficacy profile of clinically approved DAAs are very limited. Probing the altered drug metabolism among patients receiving HCV treatment with other clinical manifestations, drug-drug interactions and concurrently administered medications remain at the front position for optimizing DAA regimen. A study regarding DAA/non-DAA drug interaction recommended that simeprevir®/pegIFN/RBV may increase the risk of interstitial pneumonitis due to IFN as evinced by earlier onset of condition while comparing to conventional pegIFN/RBV treatment [42]. Another study regarding DAA/non-DAA drug interaction in a patient with recurrent HCV and cirrhosis first reported the case of seizures, possibly participated by simeprevir®/sofosbuvir®/RBV therapy [43]. Whereas risk of interstitial pneumonitis and seizures is still being evaluated, extensive cases of cardiac events in patients who received simeprevir®/sofosbuvir® and amiodarone®, an anti-arrhythmic medication having long half-life, led to extra care on the account of prescribing providers and an appendix on labels of antiviral drugs. Most cases of liver decompensation or hepatic failure from the post-approval use of simeprevir®/pegIFN/RBV or with sofosbuvir® were recorded by patients of advanced cirrhosis who were formerly at high risk for deteriorating liver function. Due to limited data available, simeprevir® is contraindicated in patients with severe cirrhosis or decompensated liver disease [44, 45]. Due to potent toxicity, the three-dimensional (3D) therapies are contraindicated in patients suffered from severe liver impairment [46, 47]. A total of 26 cases globally were significantly found to be related to 3D therapy administration with liver disorder occurring within 1–4 weeks of starting treatment [48, 49]. Moreover, it was revealed from real-world data that high incidence of primarily hypersensitivity reactions and immune system disorders may often lead to liver failure [49].

Post-marketing data obtained from HCV-/HIV-coinfected patients led to commendations for patients to stay on suppressive antiretroviral regimen while on 3D therapy due to the presence of ritonavir® (HIV-1 protease inhibitor) that can select for HIV-1 protease inhibitor resistance-associated substitutions [46–49]. Very low treatment discontinuation and AE rates were found in phase II–III study regarding sofosbuvir®-containing regimen as compared to IFN-based therapy while analyzing real-world results [50, 51]. Data depicted that two patients had to stop anti-HCV therapy earlier due to variceal bleeding and nonmedical reasons. However, therapy was well tolerated for majority of the patients (97%) [52]. Overall, Child-Pugh[2] and

[2]Score used to analyze prognosis of chronic hepatic disease, specifically cirrhosis.

Model for End-Stage Liver Disease[3] (MELD) classifications improved for majority of HCV-infected patients, therefore lessening the need for transplantation of the liver. Post-marketing data obtained from CHC patients with renal disease depicted that same treatment was safe and tolerable regardless of baseline kidney function, yet patients who received sofosbuvir®-containing regimen seemed to have higher incidence of anemia [50].

3. Anti-HCV drug combinations tolerability in distinctive population

Significant AE profile of IFN-based therapy confines the applicability of these regimens for treating recurrent hepatitis C infection in difficult-to-treat population. Intensive research struggles are being done to assess DAAs in different populations of CHC patients for whom therapeutic options are limited.

3.1. Elderly CHC patients

Recently, evidence-based retrospective cohort studies have been reported regarding safety and tolerability analysis of DAAs in elderly CHC patients. Patients (n = 244) were categorized into two groups: individuals aged under 65 years (n = 156) and patients equal to or elder than 65 years (n = 84). Treatment recommendations during the late 2012 and early 2013 were protease inhibitors in combination with pegIFN and RBV. During years of 2014 and 2015, the rest of the therapies were given to patients as approved in succession by the FDA. Different treatment combinations used were sofosbuvir®/pegIFN/RBV, sofosbuvir®/ledipasvir®/RBV, ombitasvir®/paritaprevir® /ritonavir®/dasabuvir® ± RBV, and simeprevir®/sofosbuvir®. Just three patients received telaprevir®/pegIFN/RBV, and one patient each was treated with boceprevir®/pegIFN/RBV, sofosbuvir®/ledipasvir®/RBV, and simeprevir®/sofosbuvir®/RBV combinations in cohort study [53]. With all regimen combinations, the overall end-of-treatment (EOT) response rate, defined as undetectable HCV RNA transcript after the completion of treatment, was 98.2% (n = 233) and SVR12 was 94% (n = 191). Statistically, no significant difference was found with EOT (98.8 vs. 98%) and SVR12 (93.1 vs. 94.1%) between patients aged 65 years or older and those younger than 65 years. SVR12 for DAAs/pegIFN/RBV was 98% higher than that (91.4%) obtained from IFN-free DAA regimen but was statistically insignificant. Analogous response rate was seen in patients aged 65 or older with 100% of the patients on IFN-based therapy attaining an SVR compared with only 91.07% SVR with IFN-free therapy [53]. No serious AEs were reported except two patients suffered from severe anemia. Common AEs observed in elder patients were fatigue (32.5%), anemia (19.6%), and leukopenia (11.7%) followed by thrombocytopenia (10%), skin rash (8.3%), and headache (7.9%). Leucopenia, thrombocytopenia, and anemia were observed in almost half of the patients treated with IFN/RBV. By reducing RBV dose, all patients achieved SVR12. Treatment discontinuation of RBV dose reduction did not attain statistical significance among both groups. Conclusively, on the basis of cohort studies, we can say that age is not a major factor to have an

[3]Classification used to categorize liver dysfunction in preparation for liver transplantation.

impact on SVR during treatment. Older patients did not attain higher frequency of AEs while comparing with younger patient group. Some other clinical manifestations like fibrosis, cirrhosis, ALT/AST, hemoglobin, and platelet levels may disturb the SVR in the elderly [53, 54].

3.2. Transplant recipients with chronic hepatitis C infection

HCV-related hepatic disease generally arises in patients following liver transplantation. Nearly half of the patients who have the need of liver transplant are also infected with hepatitis C infection. Viremia before transplantation is a strong predictor of virus recurrence post-transplantation. IFN-free DAA therapy has improved the long-term, posttransplantation sequel. Preeminently, DAA therapy leads to drug-drug interaction with different immunosuppressants, mainly with cyclosporine and tacrolimus. As both are substrates of CYP3A and P-glycoprotein (P-gp), treatment should be restricted to agents that are neither inducers nor inhibitors of these molecules. Currently, in a recent trial, combination of antiviral drugs (ombitasvir®/paritaprevir®/ritonavir® (25/150/150 mg q.d[4])/dasabuvir® (250 mg b.d[5])/variable RBV dose) was given for 24 weeks to transplant recipients having recurrent viral genotype 1 infection and without advanced stages of fibrosis [55]. Clinically, manageable AEs such as headache, cough, and fatigue were found to be associated with therapy. Momentarily, elevated levels of ALT and bilirubin were noticed in two patients, and nine patients were reported to have a reduced level of hemoglobin with one patient requiring erythropoietin. No significant treatment-associated abnormalities were observed in transplant recipients while comparing with those who had not undergone transplantation. Only one patient quitted treatment after 18 weeks due to memory impairment, anxiety, and rash but still cleared out the virus. Major limitation of this therapy was the need to improve tacrolimus and cyclosporine dosage [55].

In another trials, efficacy and safety of IFN-free sofosbuvir®/RBV therapy to treat CHC infection in kidney transplant recipients (n = 10) were evaluated. The effect of sofosbuvir®/RBV therapy upon calcineurin inhibitor (CNI) drug levels was also assessed. SVR12 was seemed to be maintained in all patients (100%) [56, 57]. Acute rejection graft loss was not detected during antiviral therapy. Among ten patients, seven did not exhibit significant AEs. Only one patient had symptoms of fatigue, muscle cramps, headache, and anorexia during therapy. Acute gastroenteritis was observed in one patient who recovered after 5 days. Another patient was found to have hyperuricemia with gout, but its association with sofosbuvir/RBV treatment was not recognized. All patients completed the course of treatment, and none of the patients discontinued their antiviral treatment. Significant reduction in CNI drug exposure was found during anti-HCV treatment, but none of the patients required having dose modification of CNIs [56].

3.3. HCV-/HIV-coinfected patients

HCV-/HIV-coinfected patients are at high risk for progression of liver cirrhosis and hepatic decompensation. One study assessed IFN-free ombitasvir®/paritaprevir®/ritonavir® (25/150/100 mg q.d.) and dasabuvir® (250 mg b.d.) regimen in HCV-/HIV-coinfected patients for 12 or 24 weeks [58]. This therapy was found well tolerated in study population including

[4]One tablet per day orally (Latin: quaque die).
[5]Twice a day (Latin: bis in die).

treatment-experienced and treatment-naïve patients and cirrhotic and non-cirrhotic patients. Mild-to-moderate AEs were experienced by majority of the patients (89%); however, one patient was found to have severe AEs, but none of the patients discontinued their therapy due to AEs. Along with infrequent laboratory abnormalities, no erythropoietin or transfusion was required by any patient. Ledipasvir® (90 mg q.d.) and sofosbuvir® (400 mg q.d.) treatment to HCV-/HIV-coinfected patients for 12 weeks stated mild-to-moderate AEs (77%) including fatigue, headache, and diarrhea [59]. Less than 1% of the patients were reported for laboratory abnormalities including increased levels of creatinine kinase (non-study-related), lipase, and serum glucose (in those patients who had history of diabetes or abnormal baseline glycosylated hemoglobin levels). However, no patient was reported to discontinue therapy due to AEs. Consequently, this therapy displayed less potential for clinically significant drug-drug interactions with coadministration of antiretrovirals except with the drug, tenofovir disoproxil fumarate [59].

4. Conclusion

The last few years evidently made the progress in development of successful HCV therapeutic regimens with higher clinical efficacy and inconsiderable side effects except some with severe AEs, but negligible treatment discontinuation rate was reported. Likewise, therapy duration with DAAs is markedly reduced from 6 to 12 months (pegIFN/RBV) to 3–6 months. So, development of DAAs has remarkably changed the disease management. Despite various advantages of DAA therapy, their safety profile is albeit not absolutely known. Indeed, interpreting the constraints of premarketing studies like population size and short duration trials, only during the post-approval level, it is probable to ascertain and apprehend the safety matters linked with the utilization of DAAs in real conditions. As a result, pharmacovigilance activities portray the main gadget to promote patient care and safety in comparison to the use of any medication.

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References

[1] European Association for the Study of the Liver. EASL recommendations on treatment of hepatitis C 2016. Journal of Hepatology. 2017;**66**:153-167

[2] WHO. Guidelines for the screening care and treatment of persons with chronic hepatitis C infection. Updated version, April 2016. World Health Organization. http://www.who.int/hepatitis/publications/hepatitis-c-guidelines-2016/en/ [Accessed: January 02, 2018]

[3] Webster DP, Klenerman P, Dusheiko GM. Hepatitis C. Lancet. 2015;**385**:1124-1135

[4] McKibben RA, Haberlen SA, Post WS, et al. A cross-sectional study of the association between chronic hepatitis c virus infection and subclinical coronary atherosclerosis among participants in the multicenter AIDS cohort study. The Journal of Infectious Diseases. 2016;**213**:257-265

[5] European Association for Study of Liver. The last "European Association for the Study of the Liver" guidelines on the treatment of hepatitis C. EASL recommendations on treatment of hepatitis C 2015. Journal of Hepatology. 2015;**63**:199-236

[6] Martel-laferrie V, Bichoupan K, Dieterich DT. Hepatitis C direct acting antiviral agents changing the paradigm of hepatitis C treatment in HIV-infected patients. Journal of Clinical Gastroenterology. 2014;**48**:106-112

[7] Messina JP, Humphreys I, Flaxman A, et al. Global distribution and prevalence of hepatitis C virus genotypes. Hepatology. 2015;**61**:77-87

[8] Nousbaum JB, Pol S, Nalpas B, et al. Hepatitis C virus type 1b (II) infection in France and Italy. Annals of Internal Medicine. 1995;**122**:161-168

[9] Zein NN, Rakela J, Krawitt EL, et al. Hepatitis C virus genotypes in the United States: Epidemiology, pathogenicity, and response to interferon therapy. Annals of Internal Medicine. 1996;**125**:634-639

[10] Probst A, Dang T, Bochud M, et al. Role of hepatitis C virus genotype 3 in liver fibrosis progression—A systematic review and meta-analysis. Journal of Viral Hepatitis. 2011;**18**:745-759

[11] National Institutes of Health. National Institutes of Health Consensus Development Conference Statement: Management of hepatitis C: 2002–June 10-12, 2002. Hepatology. 2002;**36**:S3-S20

[12] McHutchison JG, Lawitz EJ, Shiffman ML, et al. Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. The New England Journal of Medicine. 2009;**361**:580-593

[13] Poynard T, Colombo M, Bruix J, et al. Peginterferon alfa-2b and ribavirin: Effective in patients with hepatitis C who failed interferon alfa/ ribavirin therapy. Gastroenterology. 2009;**136**:1618-28.e2

[14] Fried MW. Side effects of therapy of hepatitis C and their management. Hepatology. 2002;**36**:S237-S244

[15] Kelleher TB. Management of the side effects of peg-interferon and ribavirin used for treatment of chronic hepatitis C virus infection. In: Post TW, editor. Up-to-date. Waltham, MA: Up-to-date Inc. http://www.uptodate.com [Accessed: February 06, 2018]

[16] Pockros PJ. Direct-acting antivirals for the treatment of hepatitis C virus infection. In: Post TW, editor. Up-to-date. Waltham, MA: Up-to-date Inc. http://www.uptodate.com [Accessed: February 06, 2018]

[17] http://www.hepctip.ca/daas/ [Accessed: February 07, 2018]

[18] Flamm SL, Lawitz E, Jacobson I, et al. Boceprevir with peginterferon alfa-2a-ribavirin is effective for previously treated chronic hepatitis C genotype 1 infection. Clinical Gastroenterology and Hepatology. 2013;**11**:81-87

[19] Biesbroeck LK, Scott JD, Taraska C, et al. Direct-acting antiviral-associated dermatitis during chronic hepatitis C virus treatment. American Journal of Clinical Dermatology. 2013; **14**:497-502

[20] Scavone C, Sportiello L, Rafaniello C, et al. New era in treatment options of chronic hepatitis C: Focus on safety of new direct-acting antivirals (DAAs). Expert Opinion on Drug Safety. 2016;**15**:85-100

[21] "FDA approves Vosevi for Hepatitis C". https://www.fda.gov/newsevents/news room/ press announcements/ucm567467.htm [Retrieved: 23rd January 2018]

[22] Dieperink E, Pocha C, Thuras P, et al. All-cause mortality and liver related outcomes following successful antiviral treatment for chronic hepatitis C. Digestive Diseases and Sciences. 2014;**59**:872-880

[23] Yoshida EM, Sulkowski MS, Gane EJ, et al. Concordance of sustained virological response 4, 12, and 24 weeks post-treatment with sofosbuvir-containing regimens for hepatitis C virus. Hepatology. 2015;**61**:41-45

[24] Lawitz E, Mangia A, Wyles D, et al. Sofosbuvir for previously untreated chronic hepatitis C infection. The New England Journal of Medicine. 2013;**368**:1878-1887

[25] Jacobson IM, Gordon SC, Kowdley KV, et al. Sofosbuvir for hepatitis C genotype 2 or 3 in patients without treatment options. The New England Journal of Medicine. 2013;**368**:1867-1877

[26] Lacombe K, Fontaine H, Dhiver C, et al. Real-world efficacy of Daclatasvir and Sofosbuvir, with and without ribavirin, in HIV/HCV coinfected patients with advanced liver disease in a French early access cohort. Journal of Acquired Immune Deficiency Syndromes. 2017;**75**:97-107

[27] Afdhal N, Reddy KR, Nelson DR, et al. Ledipasvir and sofosbuvir for previously treated HCV genotype 1 infection. The New England Journal of Medicine. 2014;**370**:1483-1493

[28] Kowdley KV, Gordon SC, Reddy KR, et al. Ledipasvir and sofosbuvir for 8 or 12 weeks for chronic HCV without cirrhosis. The New England Journal of Medicine. 2014;**370**: 1879-1888

[29] Sulkowski MS, Gardiner DF, Rodriguez-Torres M, et al. Daclatasvir plus sofosbuvir for previously treated or untreated chronic HCV infection. The New England Journal of Medicine. 2014;**370**:211-221

[30] Hézode C, Hirschfield GM, Ghesquiere W, et al. Daclatasvir plus peginterferon alfa and ribavirin for treatment-naive chronic hepatitis C genotype 1 or 4 infection: A randomised study. Gut. 2015;**64**:948-956

[31] Leroy V, Angus P, Bronowicki JP, et al. Daclatasvir, sofosbuvir, and ribavirin for hepatitis C virus genotype 3 and advanced liver disease: A randomized phase III study (ALLY-3+). Hepatology. 2016;**63**:1430-1441

[32] Forns X, Lawitz E, Zeuzem S, et al. Simeprevir with peginterferon and ribavirin leads to high rates of SVR in patients with HCV genotype 1 who relapsed after previous therapy: A phase 3 trial. Gastroenterology. 2014;**146**:1669-1679

[33] Lawitz E, Sulkowski MS, Ghalib R, et al. Simeprevir plus sofosbuvir, with or without ribavirin, to treat chronic infection with hepatitis C virus genotype 1 in non-responders to pegylated interferon and ribavirin and treatment-naive patients: The COSMOS randomised study. The Lancet. 2014;**384**:1756-1765

[34] Lok AS, Gardiner DF, Hézode C, et al. Randomized trial of daclatasvir and asunaprevir with or without PegIFN/RBV for hepatitis C virus genotype 1 null responders. Journal of Hepatology. 2014;**60**:490-499

[35] Jensen D, Sherman KE, Hézode C, et al. Daclatasvir and asunaprevir plus peginterferon alfa and ribavirin in HCV genotype 1 or 4 nonresponders. Journal of Hepatology. 2015;**63**:30-37

[36] Feld JJ, Kowdley KV, Coakley E, et al. Treatment of HCV with ABT450/r-ombitasvir and dasabuvir with ribavirin. The New England Journal of Medicine. 2014;**370**:1594-1603

[37] Zeuzem S, Jacobson IM, Baykal T, et al. Retreatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. The New England Journal of Medicine. 2014;**370**:1604-1614

[38] Hézode C, Asselah T, Reddy KR, et al. Ombitasvir plus paritaprevir plus ritonavir with or without ribavirin in treatment-naive and treatment-experienced patients with genotype 4 chronic hepatitis C virus infection (PEARL-I): A randomised, open-label trial. Lancet. 2015;**385**:2502-2509

[39] Andreone P, Colombo MG, Enejosa JV, et al. ABT-450, ritonavir, ombitasvir, and dasabuvir achieves 97% and 100% sustained virologic response with or without ribavirin in treatment-experienced patients with HCV genotype 1b infection. Gastroenterology. 2014;**147**:359-365

[40] Roth D, Nelson DR, Bruchfeld A, et al. Grazoprevir plus elbasvir in treatment-naive and treatment-experienced patients with hepatitis C virus genotype 1 infection and stage 4-5 chronic kidney disease (the C-SURFER study): A combination phase 3 study. Lancet. 2015;**386**:1537-1545

[41] Rockstroh JK, Nelson M, Katlama C, et al. Efficacy and safety of grazoprevir (MK-5172) and elbasvir (MK-8742) in patients with hepatitis C virus and HIV co-infection (C-EDGE COINFECTION): A non-randomised, open-label trial. Lancet HIV. 2015;**2**:e319-e327

[42] Tamaki K, Okubo A. Simeprevir with peginterferon and ribavirin induced interstitial pneumonitis: First case report. World Journal of Gastroenterology. 2015;**21**:1009-1013

[43] Syal G, Heldenbrand SD, Duarte-Rojo A. Seizures as a potential complication of treatment with simeprevir and sofosbuvir. American Journal of Therapeutics. 2014;**2**:1-2

[44] OLYSIO (Simeprevir) Capsules: Pacakge Insert. Titusville: Jansen Pharmaceuticals, Inc, 2013. Available at: https://www.olysio.com/shared/product/olysio/prescribing information.pdf. Published Nov 2013 [Accessed: 12 February 2018]

[45] Janssen-Cilag. OLYSIO (simeprevir) capsules, for oral use: US prescribing information 2015: 1-48. Available at: http://www.olysio.com/shared/product/olysio/prescribinginformation.pdf [Accessed: 12 February 2018]

[46] AbbVie Limited. Viekirax 12.5 mg/75 mg/50 mg film-coated tablets: EU summary of product characteristics. 2015. Available at: http://ww.ema.europa.eu [Accessed: 12 February 2018]

[47] AbbVie Limited. Exviera 250 mg film-coated tablets: EU summary of product characteristics. 2015. Available at: http://www.ema.europa.eu [Accessed: 13 February 2018]

[48] Communications FDS. FDA Warns of Serious Liver Injury Risk With Hepatitis C Treatments Viekira Pak and Technivie. Silver Spring, MD, USA: Food and Drug Administration; 2015. Available at: http://www.fda.gov/Drugs/DrugSafety/ucm468634.htm [Accessed: 13 February 2018]

[49] Technivie [Package Insert]. North Chicago, IL: AbbVie Inc.; 2015

[50] Saxena V, Nyberg L, Pauly M, et al. Safety and efficacy of simeprevir/ sofosbuvir in hepatitis C-infected patients with compensated and decompensated cirrhosis. Hepatology. 2015;**62**:715-725

[51] Jensen DM, O'Leary JG, Pockros PJ, et al. Safety and efficacy of sofosbuvir-containing regimens for hepatitis C: Real-world experience in a diverse, longitudinal observational cohort [abstract]. In: Program and Abstracts of the 65th Annual Meeting of the American Association for the Study of Livers Diseases; 7-11 November 2014; Boston, MA. p. Abstract 45

[52] Höner Zu Siederdissen C, Maasoumy B, Deterding K, et al. Eligibility and safety of the first interferon-free therapy against hepatitis C in a real-world setting. Liver International. 2015;**35**:1845-1852

[53] Sherigar JM, Gayam V, Khan A, et al. Clinical efficacy and tolerability of direct-acting antivirals in elderly patients with chronic hepatitis C. European Journal of Gastroenterology & Hepatology. 2017;**29**:767-776

[54] Synder SH, Ali B, Gonzalez HC, et al. Efficacy and safety of Sofosbuvir-based direct acting antivirals for hepatitis C in septuagenarians and octogenarians. Journal of Clinical and Experimental Hepatology. 2017;**7**:93-96

[55] Kwo PY, Mantry PS, Coakley E, et al. An interferon-free antiviral regimen for HCV after liver transplantation. The New England Journal of Medicine. 2014;**371**:2375-2382

[56] Reddy S, Sharma RK, Mehrotra S, et al. Efficacy and safety of sofosbuvir-based antiviral therapy to treat hepatitis C virus infection after kidney transplantation. Clinical Kidney Journal. 2017;**sfx112**:1-5. DOI: 10.1093/ckj/sfx112

[57] Kamar N, Marion O, Rostaing L, et al. Efficacy and safety of sofosbuvir-based antiviral therapy to treat hepatitis C virus infection after kidney transplantation. American Journal of Transplantation. 2016;**16**:1474-1479

[58] Sulkowski MS, Eron JJ, Wyles D, et al. Ombitasvir, paritaprevir codosed with ritonavir, dasabuvir, and ribavirin for hepatitis C in patients co-infected with HIV-1: A randomized trial. JAMA. 2015;**313**:1223-1231

[59] Naggie S, Cooper C, Saag M, et al. Ledipasvir and sofosbuvir for HCV in patients coinfected with HIV-1. The New England Journal of Medicine. 2015;**373**:705-713

Chapter 10

Hepatitis C Viral Dynamics Using a Combination Therapy of Interferon, Ribavirin, and Telaprevir: Mathematical Modeling and Model Validation

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Additional information is available at the end of the chapter

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Abstract

Groundbreaking new drugs called direct acting antivirals have been introduced recently for the treatment of chronic Hepatitis C virus infection. We introduce a mathematical model for Hepatitis C dynamics treated with the direct acting antiviral drug, telaprevir, alongside traditional interferon and ribavirin treatments to understand how this combination therapy affects the viral load of patients exhibiting different types of response. We use sensitivity and identifiability techniques to determine which model parameters can be best estimated from viral load data. Parameter estimation with these best estimable parameters is then performed to give patient-specific fits of the model to partial virologic response, sustained virologic response and breakthrough patients.

Keywords: hepatitis C dynamics, inverse problem, subset selection, sensitivity analysis, identifiability analysis, automatic differentiation

1. Introduction

Over 200–300 million people worldwide are infected with a virus called Hepatitis C (HCV) that affects the liver, which was discovered in 1989 [1]. It is usually spread by blood-to-blood contact via intravenous drug use, poorly sterilized medical equipment and transfusions.

Scarring of the liver and ultimately cirrhosis are just a few of the more severe complications associated with HCV [2].

Six different genotypes of HCV exist due to the highly error prone RNA polymerase with the most common being genotype 1 that has the lowest levels of response to standard treatment [3, 4]. Genotype 1 patients have about a 50% chance for sustained virologic response (SVR), while non-genotype 1 patients have about an 80% chance for SVR [5]. The clinical data used for this study were provided by the University of Sao Paulo, School of Medicine in Sao Paulo, Brazil and consist of genotype 1 patients.

One of the first treatments for HCV was 6–12 months monotherapy with interferon glycoproteins. Interferon is naturally secreted from our bodies to fight off infection and monotherapy treatment with them is associated with around 10% SVR [6]. The addition of ribavirin (RBV), a drug believed to render some of the virus non-infectious, increased SVR to around 30% [6]. RBV monotherapy is not recommended because it does not give a significant benefit to SVR [7]. Until recently, the most common therapy was a combination of pegylated Interferon (IFN) and RBV for 24–48 weeks that yielded about a 45% SVR [5, 6]. One of the major differences between IFN and standard inteferon glycoproteins is that the pegylation allows the drugs to stay in the body longer [8]. There have also been clinical trials with RBV monotherapy before and after IFN + RBV therapy as described in [9]. Recently, new drugs called direct-acting antiviral agents (DAAs) have raised the chance for SVR for HCV patients.

DAAs give an increase to about an 80% chance for SVR for genotype 1 [10]. According to the FDA, DAAs are drugs that interfere with specific steps in the HCV replication cycle by taking advantage of the biological makeup of HCV [11]. HCV is a single-stranded RNA molecule that is several nucleotides in length. During HCV's life cycle, it is translated into a polyprotein that is composed into structural and nonstructural proteins that aid in replication. During post-translational processing, DAAs called protease inhibitors block a key protease from the replication process and hinders further infection [10, 12]. Among the protease inhibitors available are boceprevir, telaprevir and simeprevir. Simeprevir is recommended over telaprevir and boceprevir because of both improved efficacy and less side effects, but telaprevir continues to be used because of its cost efficiency [13, 14].

Integration of mathematical modeling of viral dynamics with clinical data has led to further understanding of how different treatment strategies dictate viral load dynamics. One of the first mathematical models was given by Neumann et al. which attempted to describe HCV dynamics with interferon monotherapy [4]. Improvements were made to the Neumann's model to better describe different mechanisms in the liver during treatment including the regeneration of liver cells. Adjustments were also made to include the standard of care, IFN, and RBV. Some of these modifications can be found in [5, 15]. In particular, Snoeck et al. [5] had data after the end of the treatment phase so that the model can give a more accurate representation of its prediction of SVR. The introduction of DAAs has ushered in more mathematical models that include this type of therapy [16]. For example, mathematical models have been proposed using telaprevir monotherapy [17–20] and in combination

with IFN and RBV [18] that uses Bayesian feedback to estimate the parameters in the model. The challenges that come with modeling DAAs is that since they are relatively new, there is not as much data available [17]. It can be difficult to predict SVR because of a lack of data after the treatment phase ends due to how recent the drugs have been approved.

This chapter introduces a novel approach for the development of a mathematical model describing HCV dynamics given the triple-drug combination treatment of IFN, RBV, and the DAA telaprevir. In Section 2, we describe how we adapted a previously known HCV model to include telaprevir and the available clinical data. Section 3 describes the a priori analysis of sensitivity and identifiability and its incorporation into the parameter estimation problem. Section 4 gives the parameter estimation results using several patient specific clinical data including partial virologic response, sustained virologic response and breakthrough. Finally, concluding remarks are provided in Section 5.

2. Mathematical models of HCV dynamics

The original model for HCV dynamics in Neumann et al. [4] was frequently used to assess viral-load profiles after short-term treatment and is given by

$$\begin{aligned} \frac{dT}{dt} &= s - dT - (1-\eta)\beta VT, \\ \frac{dI}{dt} &= (1-\eta)\beta VT - \delta I, \\ \frac{dV}{dt} &= (1-\varepsilon)pI - cV, \end{aligned} \tag{1}$$

where T and I denote the concentrations of healthy and infected hepatocytes, and V represents viral concentration in the liver fluid. One of the key contributions of the model was the understanding of the mechanism of IFN. It was unknown whether it acted through $\eta > 0$ (i.e., inhibiting the infection of healthy liver cells) or $\varepsilon > 0$ (i.e., reducing virion production in infected cells). In [4], it is determined that it is through ε which inhibits production of the virus. The drawback to (1) is that it cannot describe patients exhibiting breakthrough, relapse, and most importantly SVR. These responses are reasons that early viral response does not uniformly predict responses in the long term. Another important aspect is the handling of viral load measurements below the lower limit of quantification (LLOQ). Previous analysis omitted the data below LLOQ, but it can contain critical information regarding long-term treatment outcome. Snoeck et al. [5] present a mathematical model for the dynamics of HCV with the drug treatment combination of IFN and RBV that attempts to address both the long-term responses and the use of the LLOQ. The model described in [5] is given by the following system of nonlinear differential equations

$$\begin{aligned}
\frac{dT}{dt} &= s + rT\left(1 - \frac{T+I}{T_{max}}\right) - dT - \beta V_I T, b \\
\frac{dI}{dt} &= \beta V_I T + rI\left(1 - \frac{T+I}{T_{max}}\right) - \delta I, \\
\frac{dV_I}{dt} &= (1-\overline{\rho})(1-\overline{\varepsilon})pI - cV_I, \\
\frac{dV_{NI}}{dt} &= \overline{\rho}(1-\overline{\varepsilon})pI - cV_{NI},
\end{aligned} \tag{2}$$

where T (uninfected hepatocytes), I (infected hepatocytes), V_I (infectious virions) and V_{NI} (noninfectious virions) are natural states (international units IU/mL). This model was adapted from a standard model of viral infection [4]. The number of uninfected hepatocytes increases each day with reproduction rate s and regeneration rate r. That number decreases each day as those hepatocytes die naturally at a rate d or infected at a rate β. The maximum number of hepatocytes per mL is T_{max}. The number of infected hepatocytes increases when the healthy liver cells are infected and when the infected cells regenerate themselves. That number decreases when they die off naturally at a rate δ. Infected hepatocytes produce both infectious and noninfectious virions at a rate p. Virions are naturally cleared at a rate c. IFN inhibits virus production while RBV renders some of the virus noninfectious. The drug efficacies of IFN and RBV are represented by ε and ρ, respectively. The bounds for IFN and RBV are $0 < \varepsilon \leq 1$ and $0 < \rho \leq 1$ where the more effective the drug is, the closer the efficacy of the drug will be to 1. Snoeck uses data that extend beyond treatment for patients so the terms $\overline{\varepsilon}$ and $\overline{\rho}$ in (2) account for the exponential decays of the efficacies of the drugs after treatment has ceased. The exponential decay of the drug efficacies is given by

$$\overline{\varepsilon} = \varepsilon e^{-k(t-t_{end})_+}, \tag{3}$$

and

$$\overline{\rho} = \rho e^{-k(t-t_{end})_+}, \tag{4}$$

where k is the efficacy decay rate, t_{end} marks the end of treatment, and

$$(a)_+ = \begin{cases} a & \text{if } a \geq 0, \\ 0 & \text{otherwise.} \end{cases}$$

The drug efficacies ε and ρ are related to the drug dosage levels by the following expressions

$$\varepsilon = \frac{\text{Dose}_{\text{PEG}}}{\text{ED}_{50_{\text{PEG}}} + \text{Dose}_{\text{PEG}}}, \tag{5}$$

and

$$\rho = \frac{\text{Dose}_{\text{RBV}}}{\text{ED}_{50_{\text{RBV}}} + \text{Dose}_{\text{RBV}}}, \tag{6}$$

where Dose_{PEG} is the weekly subcutaneous dose of IFN and $\text{ED}_{50_{\text{PEG}}}$ is the estimated weekly dose that causes 50% inhibition of virion production. Dose_{RBV} represents the daily dose of

Parameter	Value
s	$6.17 \times 10^4 \frac{\text{hepatocyte}}{\text{mL·day}}$
r	.00562 day^{-1}
β	$8.7 \times 10^{-9} \frac{\text{mL}}{\text{virion·day}}$
δ	.139 day^{-1}
c	4.53 day^{-1}
$T_{\max}$	$1.85 \times 10^7 \frac{\text{hepatocytes}}{\text{mL}}$
d	.003 day^{-1}
p	$25.1 \frac{\text{virions}}{\text{hepatocyte·day}}$
ε	.896
ρ	.4–.6
k	.0238 day^{-1}

Table 1. Typical values from [5].

RBV/kg body weight, and $\text{ED}_{50_{\text{RBV}}}$ represents the estimated daily dose in mg/kg that makes 50% of the virions noninfectious. Biologically, all state variables and parameters are non-negative. Typical values for model parameters used by Snoek et al. [5] are given in **Table 1**.

2.1. HCV model with DAA

Snoeck's model is adapted to incorporate the DAA, telaprevir. Recall that a DAA targets specific parts of the genome of the virus to inhibit both replication and infection. The hindrance of replication of the virus in the infected hepatocytes results in the virus not being produced by those cells. This means that the DAA should be implemented as part of the infection term, $\beta T V_I$, for inhibiting infection and viral production terms, pV_I and pV_{NI}, for inhibiting replication of the virus in (2). However, after simulations and analysis, it is concluded in this study that the obstruction of the infection and replication of the virus by telaprevir can be described solely as an amplifier for mitigating the production of virions alongside IFN. With this assumption, the model in [5] is modified to include the triple drug combination of IFN, RBV and telaprevir as follows:

$$\begin{aligned}
\dot{T} &= s + rT\left(1 - \frac{T+I}{T_{\max}}\right) - dT - \beta V_I T \\
\dot{I} &= \beta V_I T + rI\left(1 - \frac{T+I}{T_{\max}}\right) - \delta I \\
\dot{V}_I &= (1-\overline{\rho})(1-\overline{\varepsilon})(1-\overline{\gamma})pI - cV_I \\
\dot{V}_{NI} &= \overline{\rho}(1-\overline{\varepsilon})(1-\overline{\gamma})pI - cV_{NI},
\end{aligned} \tag{7}$$

where $\overline{\gamma}$ represents the exponential decay of the telaprevir efficacy and is defined similarly as for $\overline{\varepsilon}$ and $\overline{\rho}$ (see (3) and (4)). In [21], existence and uniqueness of solutions to this updated

HCV dynamical model were established, and a steady-state stability analysis was also performed.

2.2. Treatment schedule

The data in this research uses the treatment schedule timeline as follows (also summarized in **Figure 1**).

1. The patient is treated with the triple drug combination of IFN + RBV + telaprevir for the first 12 weeks.
2. If at 12 weeks, viral load $>$ 1000 IU/mL, then discontinue treatment. Otherwise, continue 12-week treatment of IFN + RBV.
3. If at 24 weeks, viral load $>$ LLOQ (12–15 IU/mL), then discontinue treatment. Otherwise, continue 12-week treatment of IFN + RBV.
4. If at 36 weeks, viral load $>$ LLOQ, then discontinue treatment. Otherwise, continue 12-week treatment of IFN + RBV.
5. End of treatment at 48 weeks.

Figure 1. Treatment schedule for patients used for data received from patients treated at University of Sao Paulo, School of Medicine in Sao Paulo, Brazil.

3. Subset selection

The *forward problem* refers to using a model to predict the future behavior of a system given a set of parameters. The *inverse problem*, on the other hand, is the parameterization of a model from empirical data [22–24]. There have been extensive studies about parameter selection while solving the inverse problem for biological models and other applications that can be found in [3, 22, 25–27] and references therein. In this study, we use a simple algorithm to choose a subset of parameters to be estimated from clinical data based on both sensitivity and identifiability as follows:

1. Start with the full parameter set Q.
2. Remove parameters that are not locally sensitive to attain $Q_S \subset Q$.
3. Remove parameters that are not locally identifiable from Q_S to obtain sensitive and identifiable parameter set Q_{SI}

Since these are local analyses, this procedure is repeated over a large number of parameter sets and the parameters that appear most often in Q_{SI} are the parameters that are estimated. All other parameter values are fixed to values from the literature. A biological and structural explanation for some of the fixed parameters is given in the next section.

3.1. Fixed parameters

The assumptions for fixed parameters are the same as in [5]. Since the maximum number of hepatocytes in the liver is 2.50×10^{11} and HCV RNA is distributed in plasma and extracellular fluids with a volume of $\sim 1.35 \times 10^4$ ml, then $T_{max} = \frac{2.50 \times 10^{11}}{1.35 \times 10^4} = 1.85 \times 10^7$. d is obtained from hepatocyte turnover being every 300 days and $s = T_{max} \cdot d$ can be deduced in the absence of liver disease. p is always fixed because $p(1-\varepsilon)$ appears in $\dot{V}$ and $\dot{V}_{NI}$ making p and ε, impossible to estimate uniquely. The rest of the parameters will be considered in the sensitivity analysis.

3.2. Sensitivity analysis

A sensitivity analysis is the process of understanding how the model output is affected by changes in the parameters. Sensitivity analyses are used in many branches of mathematics such as statistics, partial differential equations (PDEs), and control design [28, 29]. The parameters that give the most change in the output are said to be sensitive parameters. This is important in the *forward problem* because it allows an understanding of which parameters will give useful information. Once the parameters have been identified, a sensitivity analysis for the *inverse problem* is usually performed to determine the sensitive parameters. Parameters with minimal impact are fixed from the literature. There are two different types of sensitivity analysis: global and local. A global sensitivity analysis heavily depends on the structure of the model and quantifies how uncertainties in outputs can be apportioned to uncertainties in inputs. We refer the reader to [30] for a more comprehensive discussion. Our study uses a local sensitivity analysis that depends on the prescribed values of the parameters.

3.2.1. Sensitivity equations

The sensitivity analysis presented in this section uses a derivative-based approach. Consider the general form of an ODE model and a function z of its output

$$\begin{aligned} \frac{dy}{dt} &= f(t, y; q), \\ z &= g(t, y; q), \end{aligned} \tag{8}$$

whereby the vectors y and q contain the variables and parameters of the model, respectively. Since we are concerned with how our model output, z, is influenced by changes to our parameters, q, then we consider the partial derivative of z, $\frac{\partial z}{\partial q}$, with respect to q. One approach to computing this partial derivative is by solving the associated sensitivity equations. Differentiating both sides of the output Eq. (8) with respect to the parameter q yields

$$\begin{aligned}\frac{\partial z}{\partial q} &= \frac{\partial g}{\partial t}\frac{\partial t}{\partial q} + \frac{\partial g}{\partial y}\frac{\partial y}{\partial q} + \frac{\partial g}{\partial q}\frac{\partial q}{\partial q} \\ &= \frac{\partial g}{\partial y}\frac{\partial y}{\partial q} + \frac{\partial g}{\partial q}\end{aligned} \tag{9}$$

since $\frac{\partial t}{\partial q} = 0$ and $\frac{\partial q}{\partial q} = 1$. The two components $\frac{\partial g}{\partial y}$ and $\frac{\partial g}{\partial q}$ can be directly calculated from g, but can be cumbersome to do by hand depending on the complexity of the function g. Thus, one can employ automatic differentiation to evaluate these derivatives. Since any mathematical function can be decomposed into elementary functions, automatic differentiation numerically implements the chain rule and basic arithmetic equations repeatedly to compute the total derivative of a function with accuracy to working machine precision [31]. This is achieved with table lookups and tabulating all the functional compositions [32, 33]. An automatic differentiation (AD) code developed by Martin Fink in MATLAB was employed [34]. Finally, to calculate $\frac{\partial y}{\partial q}$, it is noted that y is continuous in t and q. Since $\frac{\partial y}{\partial q}$ exists, by taking the partial derivative with respect to q of the state equations and reversing the order of differentiation [35], we obtain

$$\begin{aligned}\frac{\partial}{\partial q}\left(\frac{dy}{dt}\right) = \frac{d}{dt}\left(\frac{\partial y}{\partial q}\right) &= \frac{\partial f}{\partial t}\frac{\partial t}{\partial q} + \frac{\partial f}{\partial y}\frac{\partial y}{\partial q} + \frac{\partial f}{\partial q}\frac{\partial q}{\partial q} \\ &= \frac{\partial f}{\partial y}\frac{\partial y}{\partial q} + \frac{\partial f}{\partial q}.\end{aligned} \tag{10}$$

Similar to $\frac{\partial g}{\partial y}$ and $\frac{\partial g}{\partial q}$, $\frac{\partial f}{\partial y}$ and $\frac{\partial f}{\partial q}$ are calculated using automatic differentiation. From (10), the sensitivity equations are given by the following coupled system of differential equations

$$\begin{aligned}\frac{dy}{dt} &= f(t, y; q), \\ \frac{d}{dt}\left(\frac{\partial y}{\partial q}\right) &= \frac{\partial f}{\partial q}\frac{\partial y}{\partial q} + \frac{\partial f}{\partial q}.\end{aligned} \tag{11}$$

Solving the sensitivity equations yields $\frac{\partial y}{\partial q}$, which, in turn, gives $\frac{\partial z}{\partial q}$ from (9).

3.2.2. Model considerations and sensitivity results

The sensitivities of each parameter are ranked to obtain which parameters are most sensitive. Since there is a large range of parameter and viral load values, each parameter, q_j, is log scaled in association with the state variable, y, that is,

$$\frac{d\log_{10}(y)}{d\log_{10}\left(q_j\right)} = \frac{q_j}{y}\frac{dy}{dq_j}$$

is considered instead of $\frac{dy}{dq_j}$. This allows a comparison of the sensitivities of each parameter using similar magnitudes. The l_2- norm is used to nondimensionalize the sensitivities over time so the following sensitivity coefficient is considered for each parameter

$$S_{ij} = \left|\left|\frac{\partial y_i}{\partial q_j}\right|\right|_2 = \left[\frac{1}{t_f - t_0}\int_{t_0}^{t_f}\left(\frac{\partial y_i}{\partial q_j}\left(\frac{q_j}{\max y_i}\right)\right)^2 dt\right]^{\frac{1}{2}}. \tag{12}$$

Eq. (12) is defined to be the relative ranking sensitivity of each variable y_i in y with respect to each individual parameter q_j.

Since the local sensitivity analysis depends on values in q, independent sets of parameters that have a log-normal distribution are created from the population-based model fit in Snoeck et al. [5]. That is, a sequence of independent parameter sets $\{q_k\}$ is generated from this distribution using the typical values from [5] as the mean. To determine pseudo-global sensitivities, a sensitivity coefficient, S_{ij}^k, is computed for each parameter in the k th parameter set. Then, if B parameter sets are to be analyzed, then an average for all the parameter sets is computed by

$$\overline{S}_{ij} = \frac{1}{B}\sum_{k=1}^{B} S_{ij}^k. \tag{13}$$

A cutoff is determined based on the ranking of the averages attained in (13). Those parameters above the cutoff are further examined in the identifiability analysis. This method is a version of what is referred to as Morris Screening in [30]. Similar to the work done here, the Morris algorithm [36] averages local derivative approximations to provide more global sensitivity measures. The difference being that the variance in the parameter sets is also considered. Here that variance would be given by

$$\sigma_{ij}^2 = \frac{1}{B-1}\sum_{k=1}^{B}\left(S_{ij}^k - \overline{S}_{ij}\right)^2. \tag{14}$$

As explained in [30], while the mean (13) quantifies the individual effect of the input on the output, the variance (14) estimates the combined effects of the input due to nonlinearities or interactions with other inputs. The reader is referred to [30, 36] and references therein for a more detailed analysis of Morris Screening. It is noted that only the marginal distributions are given in [5], so computations are ignorant of any covariances between parameters. The data that were used contain only the viral load observations. So the sensitivities of $V = V_I + V_{NI}$ are of interest. Therefore, (8) is considered where

$$y = [T\ I\ V_I\ V_{NI}]^T,$$

with output

$$z = V = V_I + V_{NI}.$$

Two different sets of time points are used during this analysis. The first and second set of time points come from the partial virologic response (PVR) case and Breakthrough case, respectively. This will provide a better illustration of sensitivities given that treatment decays in the Breakthrough case, but does not in PVR. The sensitivity rankings are given in **Figure 2** for over

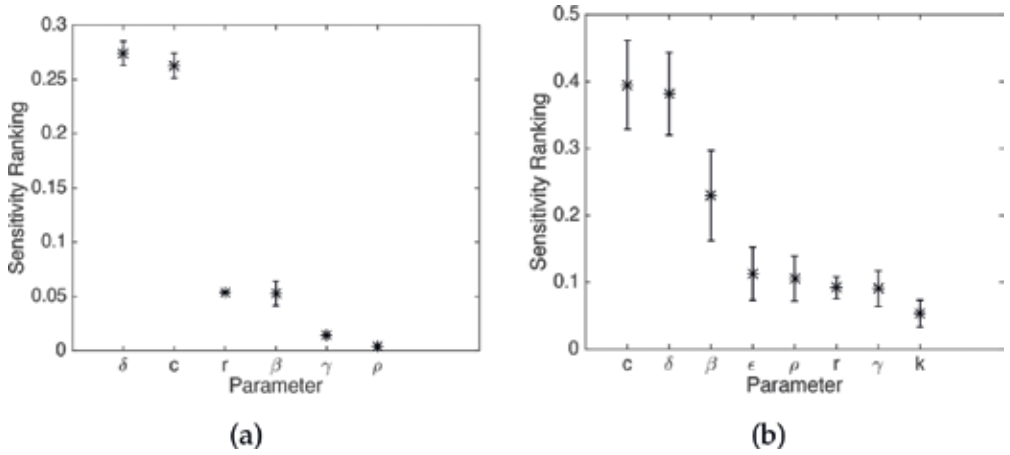

Figure 2. Sensitivity rankings using PVR (a) and Breakthrough time points (b).

2000 (a) and 400 (b) parameter sets, respectively. Error bars that are two standard deviations from the mean are included. The sensitive parameters for the PVR and Breakthrough time points are $Q_{PVR} = \{\delta, c, \beta, r, \gamma\}$ and $Q_{Brk} = \{\delta, c, \beta, r, \rho, \gamma, \varepsilon\}$, respectively. These parameters are considered in the identifiability analysis. Note that γ is always considered in the identifiability analysis due to there not being a value from the literature to fix it to for this model. It is used to determine whether it affects the identifiability of other parameters.

3.3. Identifiability analysis

After deciding which parameters are sensitive, consideration is given to understanding which sensitive parameters can uniquely be identified from the data. In this study, we employed a sensitive-based approach for local identifiability analysis. To this end, we consider the parameters contained in q which minimize the cost function

$$J(q) = \frac{1}{N}\sum_{i=1}^{N}\left(V_d^i - V(t_i;q)\right)^2,$$

with $V(t_i;q)$ denoting the model output and V_d^i denoting the corresponding data value at time point t_i for $i = 1, \ldots N$, where N is the number of data values. Similar to [37], let us assume that q^* is the minimum of this cost function. Then by using a Taylor series expansion around q^*, we obtain

$$V(t_i, q) = V(t_i; q^*) + \frac{dV(t_i; q^*)}{dq}(q - q^*) + \ldots$$

If we only consider the first two elements of $V(t_i, q)$ under the assumption that $q \approx q^*$ and substitute this expression into the cost function we find that

$$\begin{aligned} J(q) &= \frac{1}{N}\sum_{i=1}^{N}\left(V_d^i - V(t_i;q^*) - \frac{dV(t_i;q^*)}{dq}(q-q^*)\right)^2, \\ &= \frac{1}{N}\sum_{i=1}^{N}\left(\frac{dV(t_i;q^*)}{dq}(q-q^*)\right)^2, \end{aligned} \tag{15}$$

where we used the fact that q^* is the minimum of the cost function so that $V_d^i \approx V(t_i;q^*)$. Let

$$S = \frac{dV}{dq} = \begin{bmatrix} \frac{dV}{dq_1}(t_1) & \frac{dV}{dq_2}(t_1) & \cdots & \frac{dV}{dq_l}(t_1) \\ \frac{dV}{dq_1}(t_2) & \frac{dV}{dq_2}(t_2) & \cdots & \frac{dV}{dq_l}(t_2) \\ \vdots & \vdots & \vdots & \vdots \\ \frac{dV}{dq_1}(t_N) & \frac{dV}{dq_2}(t_N) & \cdots & \frac{dV}{dq_l}(t_N) \end{bmatrix}, \tag{16}$$

be an $(N \times l)$ sensitivity matrix relating to the sensitivities $\frac{dV}{dq_j}(t_i)$ of the output with $i = 1, \ldots, N$ and $j = 1, \ldots, l$, where l denotes the number of parameters. The cost function of (15) is rewritten in terms of this sensitivity matrix

$$\begin{aligned} J(q) &= \frac{1}{N}(S(q-q^*))^T(S(q-q^*)), \\ &= \frac{1}{N}(S\Delta q)^T(S\Delta q), \end{aligned}$$

where $\Delta q = q - q^*$. Rearranging $\Delta q = q - q^*$, we formulate the cost function in terms of $q^* + \Delta q$:

$$J(q^* + \Delta q) = \frac{1}{N}\Delta q^T S^T S \Delta q. \tag{17}$$

If we suppose that Δq is an eigenvector of $S^T S$ with $S^T S \Delta q = \lambda \Delta q$, then we have

$$\begin{aligned} J(q^* + \Delta q) &= \frac{1}{N}\Delta q^T(\lambda \Delta q), \\ &= \frac{1}{N}\lambda \|\Delta q\|_2^2. \end{aligned}$$

We note that if Δq is an eigenvector with eigenvalue $\lambda = 0$, then the cost function to second-order approximation is $J(q^* + h\Delta q) = 0$. The least squares cost function does not change values when moving from q^* to $q^* + h\Delta q$, with h arbitrary. Thus, the parameters are locally unidentifiable at q^*. If $S^T S$ has very small eigenvalues, this can also be a problem for parameter identification. There have been studies about how the Fisher Information Matrix ($S^T S$) can be used for parameter identification [38, 39]. For example, in [38], they search all possible parameter combinations and choose them based on the rank of the

sensitivity matrix, S, and asymptotic standard error uncertainty. We use the following algorithm as described in [39] to determine which of the parameters in our model will be unidentifiable.

1. Create the matrix $S^T S$, compute its eigenvalues, and order them such that

$$|\lambda_1| \leq |\lambda_2| \leq \cdots \leq |\lambda_n|.$$

2. If $|\lambda_1|$ is less than some threshold ε (typically taken to be 10^{-4}), we say that there is a parameter that is unidentifiable.

3. The largest magnitude component of the eigenvector Δq_1 associated with the eigenvalue λ_1 corresponds to the least identifiable parameter. Remove the corresponding column from S and repeat step 1.

After performing this procedure, we now have a set of sensitive and locally identifiable parameters to estimate. The rest of the parameters are set to "typical values" found in the literature. The identifiability algorithm is applied to all the parameter sets of sensitive parameters, Q_{PVR} and Q_{Brk}, obtained in the previous section. It is observed from **Figure 3** that the parameters in $Q_{PVR} = \{\delta, c, \beta, \gamma\}$ are identifiable at least 50% of the time and the parameters in $Q_{Brk} = \{\delta, c, \beta, \gamma, \varepsilon\}$ are identifiable at least 50% of the time. The parameters contained in Q_{PVR} and Q_{Brk} are those that will be estimated from the clinical data.

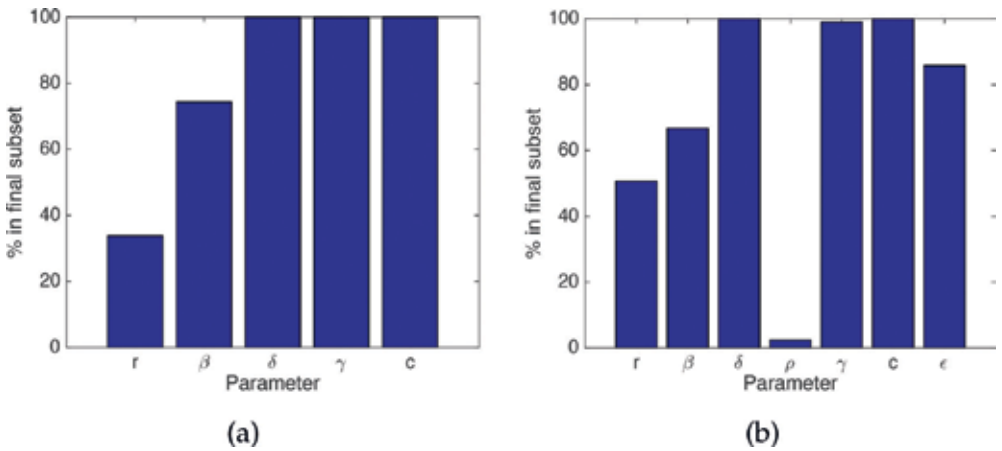

Figure 3. Final subset percentages using PVR (a) and Breakthrough time points (b).

4. Parameter estimation

The parameters in Q_{PVR} and Q_{Brk} are estimated using the weighted sum of squares of errors (WSSE) given by

$$J(q) = \sum_{i=1}^{N} w_i \left[\log\left(V_d^i\right) - \log\left(V(t_i;q)\right) \right]^2, \tag{18}$$

where w_i is the weight for the error term $\left[\log\left(V_d^i\right) - \log\left(V(t_i;q)\right)\right]$ at time t_i, V_d^i is data measurement of viral load at the i th time point and $V(t_i;q)$ is the model output with parameters q. We used both sampling and gradient based methods to minimize this function implemented in MATLAB. The model was fit to three data sets; namely, PVR, ETR (end-of-treatment response) and Breakthrough. PVR represents when the patient has an initial positive reaction to the therapy, but then the viral load rebounds during treatment and never goes below detection. ETR represents when the viral load drops below detection and does not rebound. Breakthrough represents when the patient's viral load drops below detection, but rebounds. In our data, the LLOQ is 15 IU/ml. When the data drop below the LLOQ, least squared estimation does not suffice as a statistically rigorous methodology. Instead, we employ the expectation maximization (EM) [40] to compute maximum likelihood estimates of our patient specific parameters. For a detailed description of the EM algorithm, we refer the reader to [41]. The RBV dosage depends on the patient's body weight and was sometimes modified during treatment due to different symptoms of the patients such as blood thinning. The patients experiencing PVR and Breakthrough had constant RBV dosage for the entire treatment while the patient exhibiting ETR had modified dosage. The RBV efficacy is fixed to $\rho = .1222$ from [22] for the PVR and Breakthrough patients. The efficacies for the ETR patient were modified based on time, t, in days since initial treatment and are presented in **Table 2**.

The parameters not in Q_{PVR} or Q_{Brk} are fixed to the values in **Table 3** from [5, 22]. As in [5], the infected steady state is used for the initial conditions for (7) because the patients considered had chronic infection. The values in **Table 4** are obtained after estimating the parameters in Q_{PVR} and Q_{Brk}. These estimates produce the model fits (graphs on the left) and residuals (graphs on the right) in **Figures 4–6**. It is noted that in **Figure 6**, the ETR patient's viral load goes to zero, and the residuals for censored data are set to zero.

In practice, the mathematical model is never exact (model misspecification), and the data contain noise (human errors, instrument errors). Hence, confidence and prediction intervals are used to understand the extent of uncertainty involved in estimating our parameters. In

Parameter	$t \leq 27$	$27 < t \leq 83$	$t > 83$
ρ	.5127	.3185	.219

Table 2. Patient ETR's RBV efficacies based on modified dosage.

Parameters	s	r	$T_{\max}$	d	p	ε
Values	6.17×10^4	.00562	1.85×10^7	.003	25.1	.6138

Table 3. Fixed parameter values from [5, 22].

Patient	PVR	ETR	Breakthrough
δ	$.1883 \pm .0462$	.7211	.3293
c	2.717 ± 2.724	11.67	2.089
γ	$.9987 \pm .0015$	.9999	.6575
β	$1.875 \times 10^{-5} \pm 1.688 \times 10^{-5}$	8.684×10^{-8}	2.259×10^{-6}
ε	.6138	.9829	.9875

Table 4. Values from parameter estimation for (7).

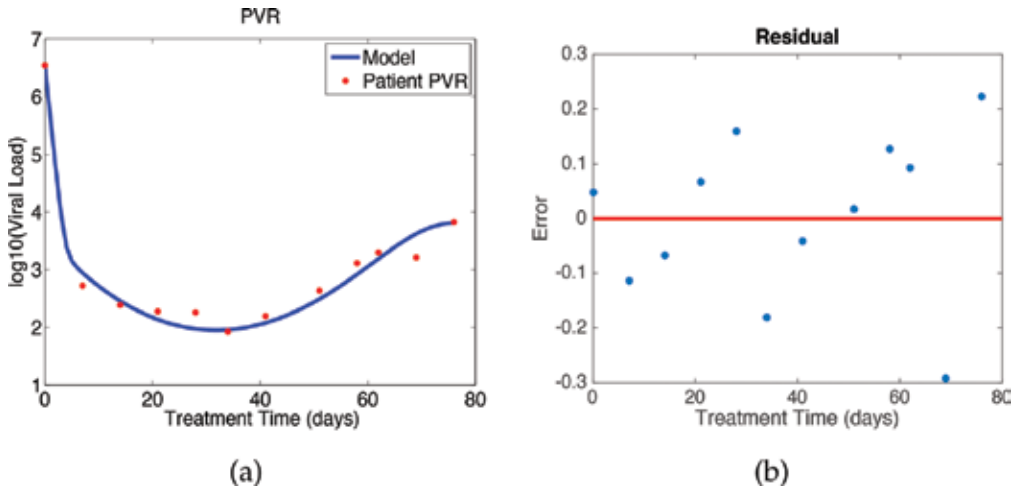

Figure 4. Viral load model fit (a) and residual plot (b) for PVR patient data.

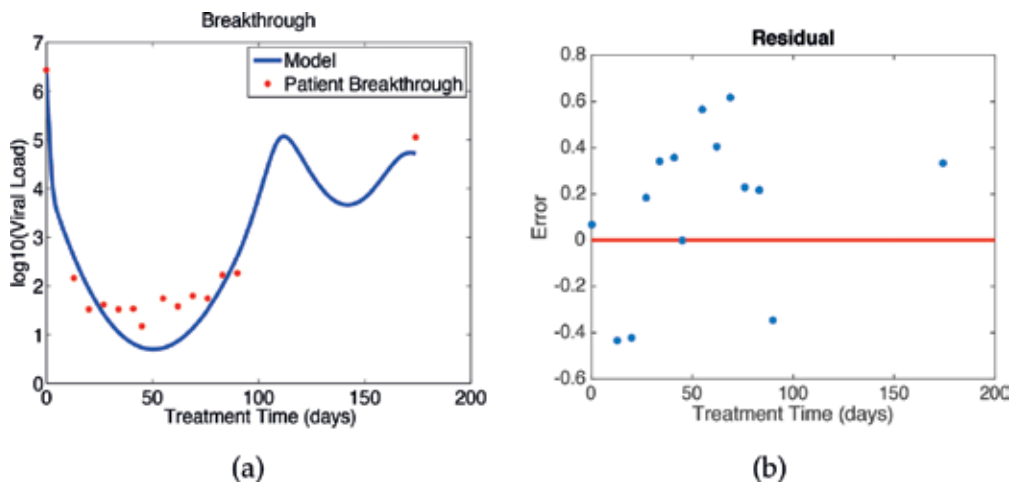

Figure 5. Viral load model fit (a) and residual plot (b) for Breakthrough patient data.

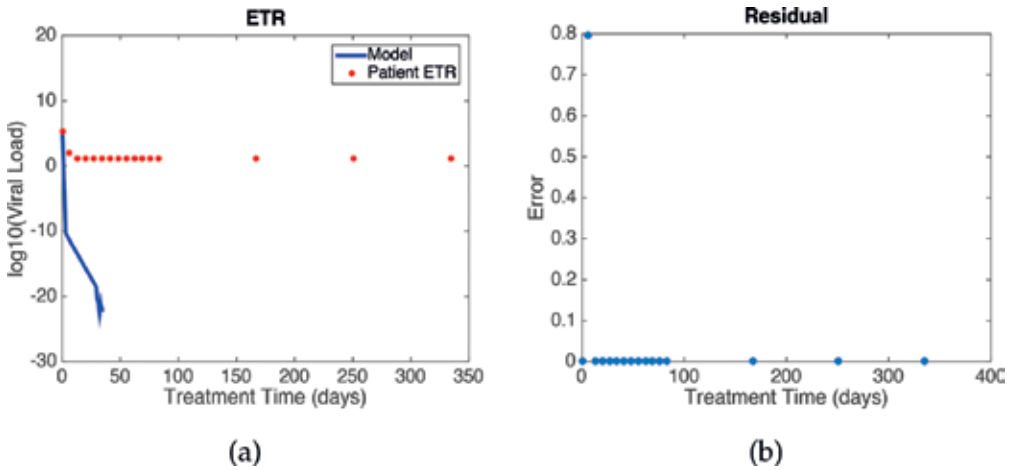

Figure 6. Viral load model fit (a) and residual plot (b) for ETR patient data.

calculating these intervals, standard errors are computed from the model predictions using the parameters that have been estimated. Moreover, 95% parameter and predictive confidence intervals and prediction intervals for the PVR parameters (attached as half-widths in **Table 4**) and predictions are calculated using the asymptotic theory outlined in [22, 27, 30, 41, 42]. The predictive confidence intervals and prediction intervals are shown in **Figure 7**.

5.1. Discussion

The higher values in c and δ in the ETR patient lead us to believe that the immune response along with the drugs has a stronger impact on the mutation and clearance of the virus. It is known that the immune response is strongly correlated with the clearance

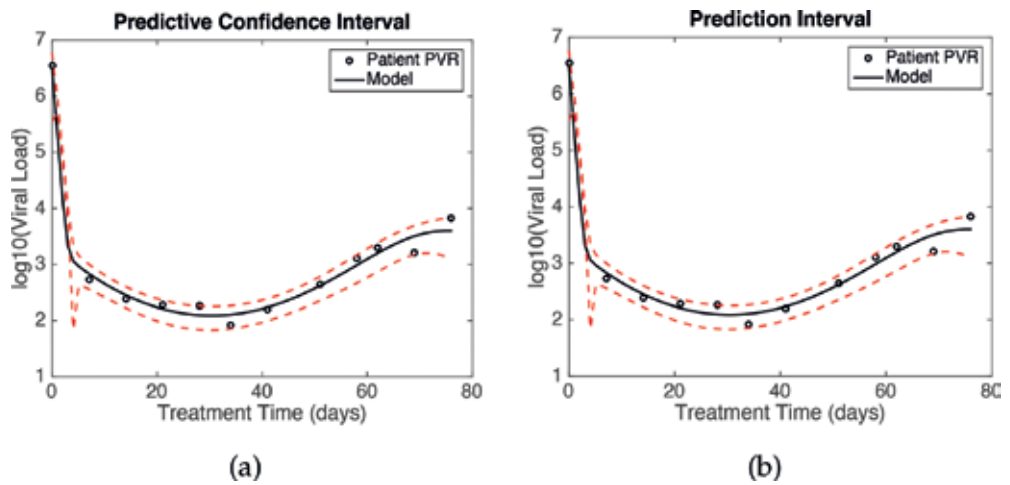

Figure 7. Predictive confidence intervals (a) and prediction intervals (b).

of the virus. Since the initial conditions of (7) are at the infected steady state, introduction of the drugs could be a mechanism to jump start the immune response. We note that even when the virus is not cleared, telaprevir still has a strong impact on viral load decay. This behavior corresponds with how powerful DAAs can be in reducing viral load even when it rebounds. The rebound could be because of mutations which are neglected in this model as stated earlier. There is a dip at around the 150th day in the Breakthrough response that is unquantifiable due to lack of information regarding the other three states or a dynamic immune response. However, this type of dip is observed in [5, 27] where data are available around this time. We conjecture that this dip is due to the immune response being stimulated by the spike in viral load and infection. The residuals in the PVR fit in **Figure 4** seem to be i.i.d. because the errors seem to be randomly distributed and are on both sides of the zero axis. This is unlike the Breakthrough fit in **Figure 5** which have most of the residuals above the zero axis. The predictive confidence intervals and prediction intervals look almost the same because the variance is very small, and the model fits the data very well. The reader is referred to [30] for further details on differences between the predictive confidence intervals and prediction intervals.

6. Conclusion

The missing data between weeks 12–24, 24–36 and 36–48 for the ETR and Breakthrough patients make parameter estimation challenging. The predictions would also be more robust if information concerning states T, I, and V_{NI} were available. These issues should be considered when making remarks about the estimations and confidence measures. DAAs were introduced in 2011, so there is not as much data available, but in the future, we hope for a larger quantity of data to make more precise estimations.

This chapter describes a model for patients with HCV who are treated with IFN, RBV, and telaprevir combination therapy. The development of this model was motivated by the desire for a model that can be validated and calibrated using sensitivity and identifiability techniques while simultaneously incorporating the new DAA, telaprevir. The model can be used to accurately describe patients exhibiting PVR, ETR, and Breakthrough.

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References

[1] Strader DB, Wright T, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis c. Hepatology. 2004;**39**:1147-1171

[2] Rehermann B. Hepatitis c virus versus innate and adaptive immune responses: A tale of coevolution and coexistence. The Jounal of Clinical Investigation. 2009;**119**:1745-1754

[3] Baraldi R, Cross K, McChesney C, Poag L, Thorpe E, Flores K, Banks H. Mathematical modeling of HCV viral kinetics, Tech. Rep. Raleigh, NC: Center for Research in Scientific Computation; July 2013

[4] Neumann A, Lam N, Dahari H. Hepatitis c viral dynamics in vivo and the antiviral efficacy of interferon-alpha therapy. Science. 1998;**282**:103-107

[5] Snoeck E, Chanu P, Lavielle M, Jacqmin P, Jonsson E, Jorga K, Goggin T, Grippo J. A comprehensive hepatitis c viral kinetic model explaining cure. Clinical Pharmacology & Therapeutics. 2010;**87**:706-713

[6] Kim AI, Saab S. Treatment of hepatitis c. The American Journal of Medicine. 2005;**118**:808-815

[7] Brok J, Gluud LL, Gluud C. Ribavirin monotherapy for chronic hepatitis c. The Cochrane Database of Systematic Reviews. 2009;(4):CD005527

[8] Veronese FM, Mero A. The impact of pegylation on biological therapies. BioDrugs. 2008; **22**:315-329

[9] Quiles-Perez R, de Rueda PM, Maldonado AM-L, Martin-Alvarez A, Quer J, Salmeron J. Effects of ribavirin monotherapy on the viral population in patients with chronic hepatitis c genotype 1: Direct sequencing and pyrosequencing of the hcv regions. Journal of Medical Virology. 2014;**86**:1886-1897

[10] Takayama K, Furusyo N, Ogawa E, Shimizu M, Hiramine S, Mitsumoto F, Ura K, Toyoda K, Murata M, Hayashi J. A case of successful treatment with telaprevir-based triple therapy for hepatitis c infection after treatment failure with vaniprevir-based triple therapy. Journal of Infection and Chemotherapy. 2014;**20**:577-581

[11] Food and D. Administration, Guidance for Industry Chronic Hepatitis C Virus Infection: Developing Direct-Acting Antiviral Drugs For Treatment. Silver Spring, MD, USA: Center for Drug Evaluation and Research; 2013

[12] Kiser JJ, Flexner C. Direct-acting antiviral agents for hepatitis c virus infection. Annual Review of Pharmacology and Toxicology. 2013;**53**:427-449

[13] Bichoupan K, Martel-Laferriere V, Sachs D, Ng M, Schonfeld EA, Pappas A, Crismale J, Stivala A, Khaitova V, Gardenier D, Linderman M, Perumalswami PV, Schiano TD, Odin JA, Liu L, Moskowitz AJ, Dieterich DT, Branch AD. Costs of telaprevir-based triple therapy for hepatitis c: $189,000 per sustained virological response. Hepatology. 2014;**60**: 1187-1195

[14] Hill A, Khoo S, Fortunak J, Simmons B, Ford N. Minimum costs for producing hepatitis c direct-acting antivirals for use in large-scale treatment access programs in developing countries. Clinical Infectious Diseases Advance Access. 2014

[15] Dahari H, Ribeiro RM, Rice CM, Perelson AS. Mathematical modeling of subgenomic hepatitis c virus replication in huh-7 cells. Journal of Virology. 2007;**81**:750-760

[16] Chatterjee A, Guedj J, Perelson AS. Mathematical modelling of HCV infection: What can it teach us in the era of direct-acting antiviral agents. Antiviral Therapy. 2012;**17**:1171-1182

[17] Adiwijaya BS, Herrmann E, Hare B, Kieffer T, Lin C, Kwong AD, Garg V, Randle JCR, Sarrazin C, Zeuzem S, Caron PR. A multi-variant, viral dynamic model of genotype 1 HCV to assess the in vivo evolution of protease-inhibitor resistant variants. PLOS Computatioal Biology. 2010;**6**:e1000745

[18] Adiwijaya BS, Kieffer TL, Henshaw J, Eisenhauer K, Kimko H, Alam JJ, Kauffman RS, Garg V. A viral dynamic model for treatment regimens with direct-acting antivirals for chronic hepatitis c infection. PLOS Computatioal Biology. 2012;**8**:e1002339

[19] Guedj J, Perelson AS. Telaprevir-based therapy increases with drug effectiveness: Implications for treatment duration. Hepatology. 2011;**53**:1801-1808

[20] Rong L, Ribeiro RM, Perelson AS. Modeling quasispecies and drug resistance in hepatitis c patients treated with a protease inhibitor. Bulletin of Mathematical Biology. 2012;**74**: 1789-1817

[21] Lankford G. Optimization, Modeling, and Control: Applications to Klystron Designing and Hepatitis C Virus Dynamics, Phd Thesis. Raleigh, North Carolina: North Carolina State University; 2016

[22] Arthur JG, Tran H, Aston P. Feasibility of parameter estimation in hepatitis c viral dynamics models. Journal of Inverse and Ill-Posed Problems. 2016

[23] Clermont G, Zenker S. The inverse problem in mathematical biology. Mathematical Biosciences. 2014;**260**:11-15

[24] Zenker S, Rubin J, Clermont G. From inverse problems in mathematical physiology to quantitative differential diagnoses. PLOS Computatioal Biology. 2007;**3**:2072-2086

[25] Banks H, Baraldi R, Cross K, Flores K, McChesney C, Poag L, Thorpe E. Uncertainty quantification in modeling HIV viral mechanics, Technical Report 16. Raleigh, NC, USA: Center for Research in Scientific Computation; December 2013

[26] Banks H, Cintron-Arias A, Kappel F. Parameter selection methods in inverse problem formulation, Technical Report 03. Raleigh, NC, USA: Center for Research in Scientific Computation; November 2010

[27] Banks H, Tran H. Mathematical and Experimental Modeling of Physical and Biological Processes. Boca Raton, FL, USA: Chapman and Hall/CRC; January 2009

[28] Banks H, Bekele-Maxwell K, Bociu L, Noorman M, Tillman K. The complex-step method for sensitivity analysis of non-smooth problems arising in biology, Technical Report 11. Center for Research in Scientific Computation; October 2015

[29] Wentworth MT, Smith RC, Banks H. Parameter selection and verification techniques based on global sensitivity analysis illustrated for an hiv model. SIAM/ASA Journal on Uncertainty Quantification. 2016;**4**:266-297

[30] Smith RC. Uncertainty Quantification: Theory, Implementation, and Applications. Philadelphia, PA, USA: SIAM; 2014

[31] Carmichael GR, Sandu A, Potra FA. Sensitivity analysis for atmospheric chemistry models via automatic differentiation. Atmospheric Environment. 1997;**31**:475-489

[32] Griewank A. On Automatic Differentiation, Mathematical Programming: Recent Developments and Applications1989. pp. 83-108

[33] Neidinger RD. Introduction to automatic differentiation and matlab object-oriented programming. SIAM Review. 2010;**52**:545-563

[34] Fink M. Automatic differentiation for Matlab: Version 1.0, June 2006. http://www.mathworks.com/matlabcentral/fileexchange/15235-automatic-differentiation-for-matlab

[35] Trench WF. Advanced Calculus. New York, NY, USA: Harper & Row Publishers; 1978

[36] Morris M. Factorial sampling plans for preliminary computational experiments. Technometrics. 1991:161-174

[37] Miao H, Xia X, Perelson AS, Wu H. On identifiability of nonlinear ode models and applications in viral dynamics. SIAM Review. Society for Industrial and Applied Mathematics. 2011;**53**:3-39

[38] Cintron-Arias A, Banks H, Capaldi A, Lloyd AL. A sensitivity matrix based methodology for inverse problem formulation, Technical Report 09. Raleigh, NC, USA: Center for Research in Scientific Computation; April 2009

[39] Quaiser T, Monnigmann M. Systematic identifiability testing for unambiguous mechanistic modeling - application to jak-stat, map kinase, and nf-kb signaling pathway models. BMC Systems Biology. 2009;**3**. Article number 50

[40] Dempster A, Rubin NLD. Maximum likelihood from incomplete data via the em algorithm. Journal of the Royal Statistical Society. 1977;**39**:1-38

[41] Attarian AR. Patient Specific Subset Selection, Estimation and Validation of an HIV-1 Model with Censored Observations under and Optimal Treatment Schedule, PhD thesis. North Carolina State University; 2012

[42] Seber GAF, Wild CJ. Nonlinear Regression, Vol. 585 of Wiley Series in Probability and Statistics. Hoboken, NJ: Wiley; 2003

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The propagation of hepatitis C from acute to chronic infection and afterward to end-stage liver diseases (hepatic fibrosis, cirrhosis, and hepatocellular carcinoma) involves a highly orchestrated series of molecular and cellular events, including a plethora of genes and cell signaling cascades. The treatment paradigms was revolutionized after the development and approval of all oral interferon-free direct-acting antivirals achieving higher sustained virologic response rates in treated individuals. This book pragmatically overviews the intricate interplay between viral and host factors during hepatitis C virus infection progression, as well as other hepatitis C-associated clinical implications. *Hepatitis C—From Infection to Cure* also provides up-to-date information about hepatitis C cures for clinicians, physicians, and healthcare providers with an ample understanding of the current treatment horizon, as well as other investigational and emerging treatment strategies. The authors with their valuable scientific contributions belong to many eminent institutes around the world and are much experienced in hepatitis C virology, pathology, and therapeutics.

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