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Hepatitis C
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Edited by Xingshun Qi and Li Yang

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Meet the editors

Dr. Xingshun Qi obtained his medical doctoral degree at the Fourth Military Medical University and completed his post-doctoral fellowship at the General Hospital of Shenyang Military Area. He is the head of the Department of Gastroenterology of the General Hospital of Northern Theater Command. His major research interests include the etiology, diagnosis, and management of liver cirrhosis, portal hypertension, portal vein thrombosis, Budd-Chiari syndrome, and hepatocellular carcinoma. He serves as a senior editorial board member of *BMC Gastroenterology*, an editorial board member of *Advances in Therapy* and *Therapeutic Advances in Gastroenterology*, and an academic editor of the *Canadian Journal of Gastroenterology and Hepatology* and *Journal of Clinical and Translational Hepatology*.

Prof. Li Yang has expertise in the diagnosis and treatment of complicated diseases of the digestive system, especially hepatic cirrhosis and autoimmune liver diseases. She is supported by several grants from the National Natural Science Foundation of China (NFSC) and other projects. She has published more than 60 peer-reviewed articles in international journals. She received a first prize Sichuan Province Science and Technology Progress Award in 2019 as a principal investigator. She is a member of the grant review boards of NFSC and the hepatology and digestive branches of the Chinese Medical Association. She is also the head of the digestive diseases group of the Hepatology Society of the Chinese Medical Association.

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Preface

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease worldwide with an estimated global prevalence of 1%. Chronic HCV infection can progress to liver fibrosis and then cirrhosis with decompensation events, and even hepatocellular carcinoma. About 1% of patients with chronic HCV infection but without liver fibrosis will develop hepatocellular carcinoma within 5 years, and approximately 13% of patients with chronic HCV infection and concomitant cirrhosis will develop hepatocellular carcinoma within 5 years. The World Health Organization (WHO) proposed an ambitious target of eliminating viral hepatitis as a public health threat by 2030, by which time there should be a 90% reduction in incident cases of HCV infection. At present, owing to the invention and widespread use of direct-acting antiviral therapy, nearly all patients with chronic HCV infection can achieve a sustained viral response. Despite this, there is still a high proportion of patients who do not know their diagnosis of HCV or who do not receive effective antiviral treatment.

This book focuses on recent advances in the management of HCV infection. It includes eight chapters written by experts worldwide that discuss the experimental mechanisms and clinical profile of the disease. The chapters approach HCV from medical, engineering, and biochemistry viewpoints.

I would like to acknowledge the authors for their contributions. I am also grateful to Author Service Manager Zrinka Tomicic at IntechOpen. I hope that this volume will prove a useful resource for those researchers and physicians interested in HCV infection.

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Section 1

Basics of Hepatitis C

Chapter 1

Hepatitis C Virus Structure and Diagnostic Methods

Müge Toygar Deniz and Sıla Akhan

Abstract

It is estimated that approximately 185 million people worldwide are infected with hepatitis C virus (HCV). The global prevalence of HCV infection is known as 2–3%. Every year, 350,000 of these patients die from complications such as cirrhosis and HCC associated with chronic hepatitis C. Therefore, early diagnosis and treatment are of great importance. It is important to reach more patients because of the use of direct-acting antivirals that provide nearly 100% permanent viral response in the treatment of HCV. In line with the 2030 target of the World Health Organization for the elimination of hepatitis C, it is important to raise awareness that HCV is a treatable disease. This chapter aims to briefly review the structure and diagnostic methods of HCV.

Keywords: hepatitis C, chronic, hepatitis C antibodies, core protein p22, hepatitis C diagnostic methods

1. Introduction

Hepatitis C virus (HCV) is a spherical, enveloped, positive-stranded, single-stranded RNA virus with a diameter of 40–80 nm. It belongs to the *Hepacivirus* genus of the Flaviviridae family [1]. The nucleocapsid with icosahedral symmetry consists of genomic RNA and many copies of core proteins. The nucleocapsid is surrounded by a host cell-derived lipid bilayer envelope in which envelope glycoproteins E1 and E2 are embedded (**Figure 1**). Core protein and E1 and E2 envelope glycoproteins are major protein components of the virion [2, 3].

1.1 HCV genome

A single precursor protein containing 3020 amino acids is synthesized from the virus's genome of approximately 9600 nucleotides [4]. The genome contains a long open reading frame (ORF) flanked by highly conserved 5'UTR (untranslated region) and 3'UTR regions. HCV genome has structural and nonstructural proteins (**Figure 2**).

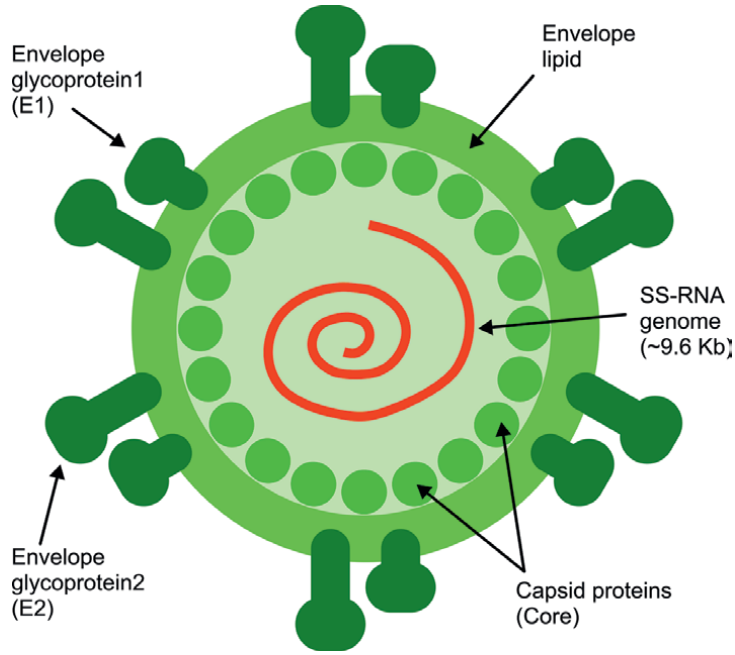

Figure 1.
HCV genome structure.

1.2 HCV proteins

1.2.1 Structural proteins

1.2.1.1 Core protein

The HCV core protein is a highly basic RNA-binding protein that makes up the viral nucleocapsid and is released as a 191 amino acid, a precursor of 23-kDa (P23). Although proteins of various sizes (17 to 23 kDa) could be detected, the 21-kDa core protein (P21) appeared to be the predominant form [5]. It has three distinct regions: the N-terminal hydrophilic domain of 120 amino acids (D1), the C-terminal hydrophobic domain of about 50 amino acids (D2), and a single peptide domain of 20 amino acids (D3) that functions as a signal transducer [6–8]. D1 serves as three predictive nuclear localization signals (NLS) and is predicted to be involved in RNA binding and nuclear localization [9, 10]. D2 is responsible for the core protein relationship with endoplasmic reticulum (ER) membranes, outer mitochondrial membranes, and lipid droplets [10, 11]. It is thought to have a role in hepatosteatosis [12]. The core protein can also be translocated to the nucleus. It regulates the transcription of cellular genes (c-myc and c-fos), protooncogenes (ras) and has apoptotic and antiapoptotic functions [13]. It also has a role in suppressing hepatitis B virus (HBV) replication. Core protein may adversely affect the inhibition of natural killer cells (NK) by increasing the expression of major histocompatibility complex (MHC) class 1 and the immune response to be formed against it by interacting with the complement receptor [14]. In addition, miR-122 inhibits HCV RNA overproduction by reducing expression [15].

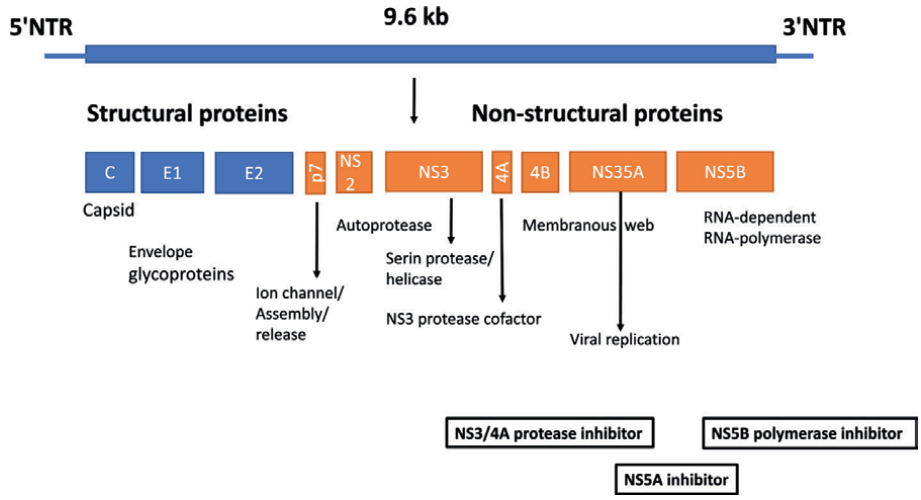

Figure 2.
HCV proteins.

1.2.1.2 Envelope glycoproteins

E1 and E2 glycoproteins are major components of the virion envelope and are required for virus entry and fusion [16]. E1 and E2 are type I transmembrane glycoproteins and have a wide variety of functions, including membrane attachment, endoplasmic reticulum localization, and virus packaging [17, 18]. The segment consisting of 30 amino acid residues in the N-terminal region of the E2 envelope glycoprotein is called HVR-1. It is the most genetically diverse of the envelope proteins. Infected individuals often produce antibodies against the HVR-1 sequence. Different subtypes are thought to cause the production of different degrees of reactive HVR-1 antibodies. Available data indicate that HVR-1 has a few neutralizing epitopes, and these epitopes are sites where mutations responsible for immune escape occur during acute and chronic infection [19–21]. However, the fact that the virus can still infect chimpanzees with the deletion of this region shows that the virus is not critical in cell entry and release.

1.2.2 Non-structural proteins

1.2.2.1 Protein p7

The p7 protein, consisting of 63 amino acids, is an integral membrane protein that acts as a calcium ion channel necessary for the efficient recruitment and release of the proliferating virus [22]. p7 is essential because mutations or deletions in its cytoplasmic portion have suppressed the infectivity of intrahepatic transfection of HCV cDNA in chimpanzees [23]. It is a therapeutic target because it is inhibited by amantadine in vitro [24].

1.2.2.2 Protein NS2

NS2 is a nonstructural transmembrane protein weighing 21–23 kDa. It forms the NS2/3 protease with the N-terminal end of the NS3 protein. The NS2/3 protease is a

zinc-dependent metalloprotease, the first of two virus-encoded proteases required for intramolecular cleavage of the HCV polyprotein [25]. It cuts the polyprotein from the NS2/NS3 junction and interacts with both structural and nonstructural proteins and plays a role in virion packaging (assembly) [26, 27].

1.2.2.3 Protein NS3

NS3 is a serine protease, RNA helicase, and nucleoside triphosphatase (NTPase) activity. NS4A is a cofactor for NS3 protease activity. NS3/4A carries additional features through its interaction with host cell pathways and proteins, which may be important in the life cycle and pathogenesis of infection; therefore it is one of the main targets of agents used for therapy [28]. It has recently been shown to antagonize the dsRNA-dependent interferon regulatory factor 3 (IRF-3) pathway, which is an important mediator of interferon induction in response to viral infection [29]. Thus, it is possible for the virus to escape from the natural cellular antiviral defense mechanisms.

1.2.2.4 Protein NS4A

The NS4A protein is a short polypeptide of 54 amino acids and the cofactor of the NS3 serine protease. It places NS3 protease on intracellular membranes through the N-terminal transmembrane segment in its structure, contributes to its correct folding by joining the N-terminal protease region, stabilizes protease against proteolytic degradation, and activates protease activity [30].

1.2.2.5 Protein NS4B

NS4B is an integral membrane protein weighing 27 kDa, which is predicted to contain at least four transmembrane domains. It is in contact with the ER membranes. NS4B has the ability to induce the formation of specialized membrane folds, also called membranous webs, that serve as scaffolds for the HCV replication complex [31].

1.2.2.6 Protein NS5A

The NS5A protein is a membrane-located phosphoprotein containing 458 amino acids. A region of NS5A consisting of 40 amino acids is called the interferon sensitivity determining region (ISDR), and mutations in this region are associated with interferon resistance [32, 33]. It plays a role in interferon resistance by inhibiting double-stranded RNA-activated protein kinase R (PKR), induced by interferon, which is an important component of the cellular antiviral and antiproliferative immune response [34]. NS5A also inhibits IRES-dependent replication through PKR inactivation [35]. NS5A is an RNA-binding protein. Positive and negative-stranded HCV RNA has the capacity to bind to its 3′ ends [36]. It also takes part in viral replication, packaging, and release of HCV particles.

1.2.2.7 Protein NS5B

NS5B has RNA-dependent RNA polymerase activity. HCV replication begins with the synthesis of complementary negative-stranded RNA from HCV's positive-stranded RNA. Then, positive-stranded RNA is synthesized from this

negative-stranded RNA. The main enzyme responsible for both of these steps is NS5B RNA-dependent RNA polymerase (RdRp) [3].

1.2.2.8 Protein ARFP/F/Core+1

The HCV F/ARFP/Core+1 protein, where ARFP indicates alternative reading frame protein, F indicates frameshift, and Core+1 indicates its position, suppresses type I and III interferon induction, presumably mediated through the retinoic acid-inducible gene I (RIG-I) signaling pathway, together with other viral factors. It plays a role in the modulation of host immunity [37]. It is thought to play a role in the pathogenesis of hepatocellular carcinoma (HCC) [38].

1.3 HCV replication

The entry of HCV into the cell occurs in a pH and clathrin-dependent manner. HCV primarily binds to glycosaminoglycans (GAG) and low-density lipoprotein receptor (LDL-R). Following its binding to LDL-R and GAGs, it binds to CD81 and scavenger receptor class B type I (SR-BI) via the HVR1 region of the E2 envelope glycoprotein [39, 40]. SR-BI belongs to the CD36 family and is a high-density lipoprotein (HDL) receptor [41]. Following the interaction with HCV, CD81, and SR-BI, it is directed to tight junctions and binds to tight junction proteins claudin-1 and occludin [42–44]. The virus then undergoes clathrin-dependent endocytosis. The virus, which enters the cell with the endosome, reaches the cytoplasm as a result of the conformational change and fusion in the envelope proteins, as the pH in the endosome decreases. The nonenveloped capsid that enters the cytoplasm opens and viral RNA is released into the cytoplasm. The translation is initiated by IRES on the smooth ER membrane and is cleaved translationally and posttranslationally by the HCV polyprotein, the NS2/3 and NS3/4A proteases of HCV, and the cellular proteases of the host. Local pH changes also convert envelope proteins into their three-dimensional shape. HCV replication is catalyzed by the NS5B RNA-dependent RNA polymerase. Replication ends with the fusion of the virion with the endosomal membrane (**Figure 3**). After replication of the RNA, the viral particles are packaged, the virion matures and is released from the host cell [45, 46]. In addition to the liver, HCV replicates in peripheral blood mononuclear cells, lymphoid follicles, and bone marrow [45, 46]. HCV has mostly (more than 85%) the same secretion pathways in serum as lipoproteins, and these structures are termed "lipoviral particles" (LVP) [47].

1.4 Epidemiology of hepatitis C virus

Approximately 185 million people worldwide are infected with HCV. The global prevalence of infection is known as 2–3% [30, 48]. The most affected regions are Eastern Mediterranean and European regions, with a prevalence of 2.3 and 1.5%, respectively. Egypt has a reported seroprevalence of about 14.7% which is the highest in the world. Substantial regional differences exist in the distribution of HCV genotypes in the world [49]. Every year, 350,000 of these patients die from complications, such as cirrhosis and HCC, associated with chronic hepatitis C (CHC) [50].

1.5 Methods of diagnosis of hepatitis C

Tests used for diagnosis of hepatitis C disease can be divided into serological tests that measure antibodies to hepatitis C and molecular analyzes that measure

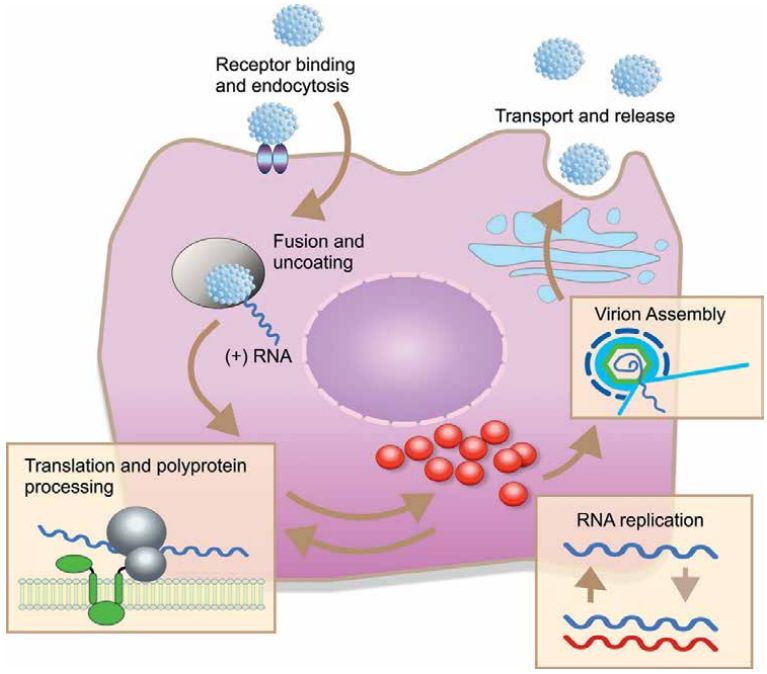

Figure 3.
HCV replication.

or detect HCV RNA. Genotype test, biochemical parameters showing fibrosis in serum and liver biopsy are other tests that should be done because they show the response to treatment and prognosis. European Association for the Study of the Liver (EASL) and Centers for Disease Control and Prevention (CDC) currently recommend HCV RNA testing together with the detection of anti-HCV antibodies in the diagnosis of HCV infection [51, 52]. The groups that should be screened for HCV are listed below [53].

1. Those born between 1945 and 1965
2. People who use intravenous drugs
3. Those with HIV infection
4. Patients with hemophilia who received factor concentrate before 1987
5. Patients on long-term hemodialysis treatment
6. Transfusion or transplant recipients
7. Blood or organ recipients from donors with HCV infection
8. Children born to mothers with HCV infection
9. HCV-contaminated needle stick or mucosal exposure

10. Those who have a sexual partner with HCV infection

11. Those with extrahepatic manifestations

12. Those with abnormal liver function tests

The diagnosis of HCV infection is made by detecting anti-HCV antibodies by ELISA and then demonstrating HCV RNA by molecular methods. Quantitative measurement of HCV RNA is used to confirm the diagnosis and the detection limit should be 25 IU/ml or less. In resource-limited locations or where HCV RNA testing is not available, HCV core antigen testing is a viable alternative. Despite the high specificity (>99%) of the anti-HCV test, positivity can be seen in previous acute or chronic hepatitis and false positive results are not uncommon. Especially pregnant women are patient groups that can be false positives in immunological or hematological disease states. At the same time, false negative results can be detected in dialysis patients, transplant patients, HIV-positive patients, and patients with severe immunodeficiency [54, 55]. In such cases, the result should be confirmed by HCV RNA testing. In addition, it should be considered that anti-HCV becomes positive 2–6 months after exposure in patients with acute hepatitis C, and HCV RNA should be requested for diagnosis.

Reactive antibody and negative HCV RNA: A negative HCV RNA test confirms the absence of chronic HCV. False-negative results of RNA are unlikely when sensitive qualitative or quantitative assays that can measure <50 IU/ml are used. As a result, this occurs either after false positive antibody tests for technical reasons or after successfully treating chronic hepatitis C infection. Other rare causes are the transmission of antibodies from a mother with HCV to her baby resulting from antibodies passively passed through blood transfusion.

1.5.1 Detection of anti- HCV antibodies

Antibodies to HCV can be detected by a variety of business models, including standardized laboratory immunoassays, rapid point-of-care immunoassays, and self-administered home testing.

1.5.1.1 Standard immunoassay test

To detect anti-HCV antibodies in serum and plasma, most clinical laboratories use tests that give a positive signal as a result of an enzymatic reaction (EIA or ELISA) or light emission (chemoluminescent assay). These tests provide many advantages, including ease of use, low variability, ease of automation, and relatively low cost. There are several immunoassay tests that target different viral antigens and detect antibodies with varying accuracy. Third-generation EIAs (EIA-3) currently in routine use usually detect antibodies against antigens originating from the core, NS3, NS4, and NS5 proteins. These tests have very high sensitivity and high specificity [56, 57]. dAnti-HCV EIA tests become positive as early as 8 weeks after exposure, and most patients are seroconverted two to 6 months after exposure [58, 59].

1.5.1.2 POCT (point of care test), rapid immunoassay tests

Several rapid tests have been developed for HCV antibodies with performance comparable to standard laboratory-based immunoassays. These tests can be

performed on venous blood, fingertip blood, serum, plasma, and oral fluid, and results are usually available in less than 30 minutes. The tests are designed as point-of-care testing to provide more opportunities for HCV testing outside of traditional clinical settings [60].

In the United States, a rapid test (OraQuick HCV Rapid Antibody Test) has been approved by the US Food and Drug Administration (FDA). The test gives results quickly using venous blood, fingertip blood, and saliva. The data suggest that the sensitivity and specificity of the test are equivalent to the EIA test [61].

1.5.1.3 Self-assembled tests

An over-the-counter antibody testing kit (hepatitis C check) has been approved by the FDA. A sample is sent to the lab and results are reported within 4–10 business days.

1.5.1.4 Recombinant immunoblot analysis (RIBA)

Recombinant immunoblot analysis (RIBA) is a test that detects HCV antibodies with similar sensitivity but higher specificity as the second-generation EIA. Where RIBA is available, it can help differentiate between a previous infection (RIBA positive) and a false positive antibody test (RIBA negative) in people with reactive antibody and negative HCV RNA testing.

1.5.2 HCV RNA detection

HCV RNA detection and measurement are essential tools in the diagnosis and management of individuals with chronic HCV infection. HCV RNA measurements are used to confirm the presence or absence of infection and to quantify the amount of HCV RNA present and can be used to guide decisions regarding the duration of treatment with certain regimens. Various methods can be used to detect and quantify HCV RNA and have varying levels of sensitivity. These include polymerase chain reaction (PCR)-based methods, transcription-mediated amplification (TMA), and branched DNA testing.

1.5.3 HCV core antigen test (HCV cAg)

Various immunoassays have been developed to detect the HCV core (HCV cAg) protein, which is a component of the viral particle [62, 63]. In resource-limited populations where nuclear acid testing (NAT) is not available, World Health Organization (WHO) guidelines recommend using an HCV cAg test to confirm viremia [64]. It is a test based on the detection of core antigens of HCV using specific monoclonal antibodies and works with the ELISA or chemoluminescent immune assay (CLIA) method [65]. The lower detection limit varies depending on the HCV genotype but is 500–3000 IU/ml [66, 67]. Although HCV RNA tests have a low detection level of 5–15 IU/mL, approximately 90% of HCV RNA positive samples are above 10,000 IU/mL, which is within the sensitivity range of the HCV core antigen test. Therefore, the next step following the anti-HCV positive screening test may be HCV core antigen detection. In a systematic review of studies evaluating the accuracy of these tests, the most studied analyses (Abbott ARCHITECT HCV Ag test and Ortho HCV Ag ELISA) detected HCV viremia in approximately 93 and 99%, respectively [62].

1.6 Additional examinations

Determining the extent of liver damage by liver biopsy is important for a newly diagnosed chronic HCV patient. It determines the duration of treatment and the treatment regimen. In addition, knowing the additional diseases of the patient is important in terms of predicting the response to treatment, urgency of treatment, and treatment complications. Basal laboratory tests, such as serum aminotransferases, bilirubin, prothrombin time, albumin, complete blood count, glucose, renal functions, urine analysis, 25 hydroxy vitamin D, and pregnancy test, should be requested. In addition, other causes of chronic liver disease, such as autoimmune hepatitis and hemochromatosis, should be investigated in patients with elevated liver function tests. Hepatitis A, B, and HIV serology should be screened and vaccination should be offered if necessary.

Since kidney diseases, such as mixed cryoglobulinemia and membranoproliferative glomerulonephritis, can be seen in HCV, patients should be examined for proteinuria, hematuria, hypertension, and kidney function tests. Additional tests for cryoglobulinemia, complement levels, and rheumatoid factor should be requested, and it should be kept in mind that renal biopsy may be required for diagnosis when significant proteinuria or unrecovered renal functions are observed.

Since HCV genotype is important in determining which treatment regimen will be given for how long, at what dose, and in determining the response to treatment, it is another routine test.

2. Conclusion

In conclusion, since HCV is usually asymptomatic, it is important to know and disseminate diagnostic methods in order to reach more people to be treated. Recently, with the use of DAAs for treating HCV, the success rate has exceeded 90%. The implementation of these strong therapies has reduced the role of monitoring therapy with HCV-RNA tests. Since HCV cAg detects viremia, it can be used especially in populations with limited resources where nuclear acid test is not available.

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Chapter 2

HCV Phylogenetic Classification

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Abstract

HCV's considerable genetic variability, which exists at various levels across viral populations in individual infected individuals at any given moment and during evolution, is a distinguishing feature of the virus. Because of this, it was discovered in 1993 through phylogenetic analysis of incomplete HCV sequences from several patient isolates worldwide that the virus could be divided into six major genotypes with significant subtypes. Based on a study of full-length ORF sequences, this categorisation was later verified. A seventh significant genotype has been identified, albeit only detected in a few people. An eight genotype has also been recently identified. The number of published ORF sequenced HCV isolates has dramatically increased because of breakthroughs in sequence analysis tools. This chapter seeks to identify the 7 main genotypes and 93 additional subtypes of HCV.

Keywords: HCV, genotype, subtypes, sequence, isolate

1. Introduction

Much emphasis has been paid to the phylogeny and molecular evolution of HCV over the last two decades. These events have far-reaching ramifications for viral taxonomy and disease epidemiology, as well as pathogen control and tracing. Furthermore, they are critical components in diagnoses, treatment regimen selection, and patient follow-up schedules, as well as vaccine development. In this paper, we outline the current perspective of HCV phylogeny and examine the genesis, distribution, and clinical significance of HCV genotypes. In addition, we describe the available evidence on HCV molecular evolution in the context of host-virus interplay at various biological levels, during the disease course, and after treatment.

The genetic diversity of the hepatitis C virus (HCV) is quite significant, and the variety of HCV genotypes and subtypes is growing. HCV was formerly divided into 7 different genotypes that varied by more than 30% at the nucleotide level [1]. Four individuals from the Indian state of Punjab who were epidemiologically unrelated were recently found to have the unique HCV genotype 8, which forms a different phylogenetic group from previously reported genomes [2]. Subtypes of genotypes having a sequence divergence of less than 15% are further subdivided [1]. HCV genotypes 1, 2, and 3 are present globally, albeit their distribution varies depending on the region [3].

The most common HCV genotype worldwide (46%) is genotype 1. While subtypes 1a and 1b of the HCV virus are more common in North America, Europe, and Australia, subtype 1b infection affects 73% of HCV-infected people in Japan. Regardless of location, persons who inject drugs (PWIDs) have a disproportionately large distribution of genotype 3, the second most common genotype in the world (30%) and is mainly found in South Asia. HCV genotype 4 infections are primarily prevalent in Africa and the Middle East, while genotypes 5 and 6 are only found in Southern Africa and Southeast Asia, respectively [4]. Multiple subtypes make up genotypes 1, 2, 3, 4, and 6, exhibiting a high genetic diversity level. HCV genotype 7a was discovered in a patient from the Democratic Republic of the Congo in 2006. Another patient from the same area was later found to have genotype 7b infection [1, 5]. Only one subtype has been documented for genotype 5 and the recently discovered genotype 8.

It is essential to describe novel subtypes and comprehend the potential effects of novel subtypes on treatment success, given the substantial genetic variety of HCV, both at the genotype and subtype levels. This chapter reviews a thorough investigation of viral diversity and sequence variation across genotypes to uncover uncharacterised subgroups and their impact on treatment outcomes.

2. Confirmed genotypes and subtypes revision

From the 18 mentioned in 2005 (1), there are now more verified genotypes and subtypes: 67 in 2013 (2), 86 in 2017, 90 in May 2019, and 93 in March 2022 (**Table 1**). The first virtually entire genome sequence of the HCV was published in 1989. Prior to this discovery, HCV had developed and spread unnoticed across the human population for hundreds of years, giving birth to a diverse range of endemic and epidemic isolates capable of causing chronic liver disease. It quickly became apparent that isolates from various people or nations had significant genetic variability [63]. This variation was compiled after thorough research and surveys by organisations from around the world, and variants were assigned as genotypes and subtypes in a consensus classification and nomenclature system. Official standards were also established for the assignment and naming of future variants [6]. Phylogenetic groups distinct from previously described sequences, at least three epidemiologically unrelated isolates, one or more complete coding region sequences, and the exclusion of intergenotypic or intersubtypic recombination, regardless of whether the components were classified, are all requirements for genotype and subtype assignments [1]. Using these criteria validated the identification of six unique genotypes with 18 subtypes. In addition, 58 subtypes were temporarily designated, awaiting the discovery of further isolates or complete sequencing for the coding area. This consensus on nomenclature was mirrored by the creation of several curated databases, including the Los Alamos HCV Sequence Database, the euHCVdb [64], and the Hepatitis Virus Database (http://s2as02.genes.nig.ac.jp/), which organised HCV sequences as they became available and indicated which genotypes and subtypes were confirmed or provisionally assigned. A proposal to standardise the numbering of HCV concerning genotype 1a isolate H77 was made concurrently (AF009606) [65].

SSEv1.1 [66] and Muscle v3.8.31 [67] were used to align with unique HCV entire or nearly complete coding area sequences from NCBI Genome (969 sequences, http://www.ncbi.nlm.nih.gov/genome) and the Los Alamos HCV sequence database (1364 sequences >8000 nt from http://hcv.lanl.gov/content/index). Seven significant

Genotype[1]	Locus/Isolate(s)[2]	Accession number(s)	Reference(s)
Genotype 1			
1a	HPCPLYPRE, HPCCGAA	M62321, M67463	[7, 8]
1b	HPCJCG, HPCHUMR	D90208, M58335	[9, 10]
1c	HPCCGS, AY051292	D14853, AY051292	[11]
1d	QC103	KJ439768	[12]
1e	148,636	KC248194	[13]
1 g	1804	AM910652	[14]
1 h	EBW443, EBW9	KC248198, KC248199	[13]
1i	QC181	KJ439772	[12]
1j	QC329	KJ439773	[12]
1 l	136,142, EBW424	KC248193, KC248197	[13]
1 m	QC196, QC87	KJ439778, KJ439782	[12]
1n	QC113, QC74	KJ439775, KJ439781	[12]
1o	QC316, DE/17-0414	KJ439779, MH885469	[12]
Genotype 2			
2a	HPCPOLP, JFH-1	D00944, AB047639	[15, 16]
2b	HPCJ8G, JPUT971017	D10988, AB030907	[17, 18]
2c	BEBE1	D50409	[19]
2d	QC259	JF735114	[20]
2e	QC64	JF735120	[20]
2f	ZS542, GZ98799	KC844042, KC844050	[21]
2i	D54	DQ155561	[22]
2j	C1799, QC232	HM777358 JF735113	[20]
2 k	VAT96	AB031663	[23]
2 l	MRS89, PTR7904	KC197235, KC197240	[24]
2 m	QC178, BID-G1314	JF735111, JX227967	[20]
2q	963,852	FN666428, FN666429	[25]
2r	QC283	JF735115	[20]
2 t	MRS40	KC197238	[24]
2u	QC182	JF735112	[20]
2v	495-05	MW041295	[26]
Genotype 3			
3a	HPCEGS, HPCK3A	D17763, D28917	[27, 28]
3b	HPCFG	D49374	[29]
3d	NE274	KJ470619	[30]
3e	NE145	KJ470618	[30]
3 g	BID-G1243, QC260	JX227954, JF735123	[31]
3 h	QC29	JF735121	[31]
3i	IND-HCV, BID-G1244	FJ407092, JX227955	[32]

Genotype[1]	Locus/Isolate(s)[2]	Accession number(s)	Reference(s)
3 k	HPCJK049E1, QC105	D63821, JF735122	[31]
Genotype 4			
4a	ED43	Y11604	[33]
4b	QC264	FJ462435	[34]
4c	QC381	FJ462436	[34]
4d	03-18, QC382	DQ418786, FJ462437	[34]
4f	IFBT88, PS6	EF589161, EU392175	[35]
4 g	QC193	FJ462432	[34]
4 k	PS3, QC383	EU392173, FJ462438	[34, 35]
4 l	QC274	FJ839870	[34]
4 m	QC249	FJ462433	[34]
4n	QC97	FJ462441	[34]
4o	QC93	FJ462440	[34]
4p	QC139	FJ462431	[34]
4q	QC262	FJ462434	[34]
4r	QC384	FJ462439	[34]
4 s	QC361	JF735136	[36]
4 t	QC155	FJ839869	[34]
4v	CYHCV073, BID-G1248	HQ537009, JX227959	[37]
4w[3]	P212, P245	FJ025855, FJ025856	[38]
Genotype 5			
5a	EUH1480, SA13[4]	Y13184, AF064490	[39]
Genotype 6			
6a	EUHK2,6a33	Y12083, AY859526	[40]
6b	Th580	D84262	[41]
6c	Th846	EF424629	[42]
6d	VN235	D84263	[41]
6e	GX004	DQ314805	[43]
6f	C-0044	DQ835760	[44]
6 g	HPCJK046E2	D63822	[45]
6 h	VN004	D84265	[41]
6i	Th602	DQ835770	[44]
6j	Th553	DQ835769	[44]
6 k	VN405	D84264	[41]
6 l	537,796	EF424628	[42]
6 m	B4/92	DQ835767	[44]
6n	KM42, D86/93	DQ278894, DQ835768	[44]
6o	QC227	EF424627	[42]
6p	QC216	EF424626	[42]

Genotype[1]	Locus/Isolate(s)[2]	Accession number(s)	Reference(s)
6q	QC99	EF424625	[42]
6r	QC245	EU408328	[46]
6 s	QC66	EU408329	[46]
6 t	VT21, D49	EF632071, EU246939	[47, 48]
6u	D83	EU246940	[47]
6v	NK46, KMN-02	EU158186, EU798760	[49]
6w	GZ52557, D140	DQ278892, EU643834	[50]
6xa[5]	DH012, DH028	EU408330, EU408332	[51]
6xb	TV476, VN110	JX183552, KJ567645	[52, 53]
6xc	TV520	KJ567651	[53]
6xd	L23, L347	KM252789, KM252790	[54]
6xe	DH027, KM98	JX183557, KM252792	[52, 55]
6xf	VN214, TV469	KJ567647, KJ567646	[53]
6xg	KS27, KS81	MH492360, MH492361	[54]
6xh	1350-1	MG879000	[56]
6xi	KM35, YNKH261	JX183549, MZ504973	[52, 57]
6xj	KM45, YNKH298a	DQ278891[6], MZ171127	[58, 59]
Genotype 7			
7a	QC69	EF108306	[60]
7b	BAK1	KX092342	[61]
Genotype 8			
8a	GT8-1	MH590698	[62]

An alignment of these genotypes/subtypes is be found at http://hcv.lanl.gov/content/sequence/NEWALIGN/align.html.[1]*Gene/subtype names that have been unanimously suggested. Two sequences of an HCV genotype have been listed where more than one is available, with the sequences prioritised by (i) publication date, or (ii) submission date when unpublished.*
[2]*Locus (or isolate name if locus is the same as the accession number).*
[3]*Previously described as 4b [38].*
[4]*A chimpanzee infected experimentally with (human-derived) isolate SA13 had its sequence taken from the acute phase plasma.*
[5]*Previously described as 6u [47].*
[6]*Previously described as 6 k [58].*

Table 1.
Confirmed HCV genotypes and subtypes (march, 2022) (adapted from Simmonds et al. [6]).

phylogenetic groups corresponding to genotypes 1 through 8 are revealed by phylogenetic analysis of sequences that comprise >95% of the coding area (**Figure 1**). 100% of bootstrap replications support clustering of the constituent subtypes within these genotypes.

According to the consensus criteria, confirmed subtypes (indicated by a letter after the genotype) need sequence data from at least two other isolates in core/E1 (>90% of the sequence corresponding to positions 869 to 1292 of the H77 reference sequence [accession number AF009606] numbered according to reference [65]) and NS5B (>90% of pos (**Table 1**) [6].

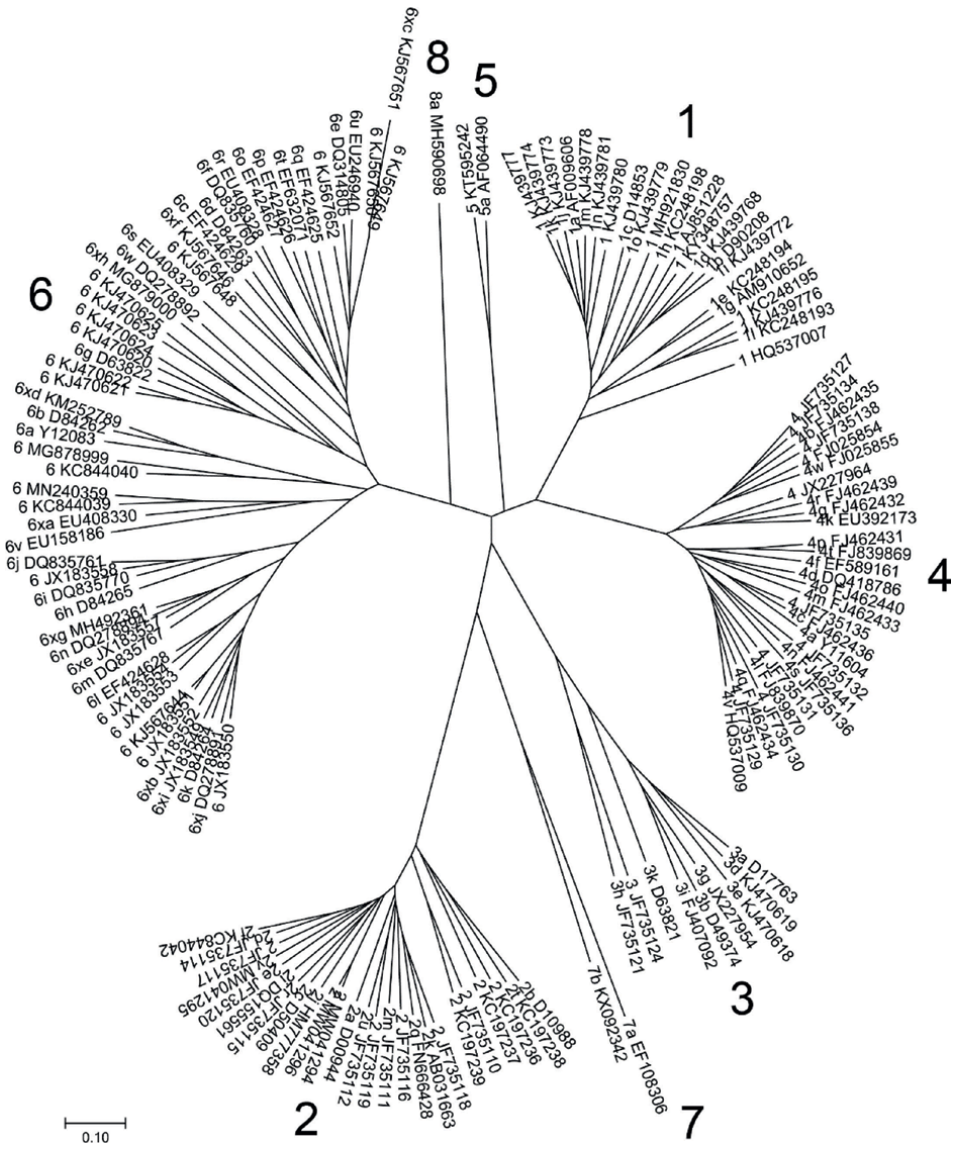

Figure 1.
The typical full coding area sequences of HCV are arranged in this phylogenetic tree. As proposed in the 2005 consensus proposal (1), for an HCV isolate to be considered as a new confirmed genotype or subtype, a complete coding region sequence that: (a) forms a distinct phylogenetic group from previously described sequences, (b) is represented by at least three epidemiologically unrelated isolates, and (c) does not represent a recombinant between other genotypes or subtypes should be obtained.

Analysis of the many possible subtypes that have been sequenced (**Figure 2**) lends credence to using a 15% criterion throughout the coding area. Except for the distances of 14 and 14.2% between JX227963 and two subtype 4 g sequences, this shows significant and regular gaps in the pairwise distances within and between each genotype's subtype distribution, which were dispersed as follows.: genotype 1: 12.9–17.0%, genotype 2: 13.1–17.6%, genotype 3: 12.5–19.6%, genotype 4: 12.7–15.3%, and genotype 6: 9.9–14.9% (with the exception of the 13.1–13.7% between EU246931

and three subtype 6e sequences). Therefore, a substantial distinction between isolates that differ by 13% throughout their full coding area sequences (members of the same subtype) and those that differ by >15% can be determined for all genotypes with very few exceptions (different genotypes or subtypes). Sequences that are not currently represented by three or more independent isolates of recognised HCV subtypes but are different from any of those subtypes are included in this chapter. It is uncertain if the reported outliers result from different epidemiological histories or technological issues [1].

The eight genotypes that have been confirmed (described in **Figure 1**) consist of 93 established subtypes, 13 subtypes that have been allocated tentatively, and 47 subtypes that have not yet been assigned [68]. These tables are available on the ICTV website at http://talk.ictvonline.org/links/hcv/hcv-classification.html and will be updated on a regular basis by the authors with data from other resources, such as typing tools, HCV databases (http://hcv.lanl.gov/; http://euhcvdb.ibcp.fr/euHCVdb/), and subtyping tools (e.g., On the ICTV Website and at http://hcv.lanl.gov/content/sequence/NEWALIGN/align.html.

A few variations with contradictory assignments were discovered during the production of these tables. Isolates P026, P212, and P245 (FJ025854-6) are classified as subtype 4b [38], although their full coding region sequences only share 85% similarity with isolate Z1 (U10235, L16677), which is provisionally designated as 4b [69] and more closely linked to isolate QC264's core/E1 (FJ46243516 [34]). A third isolate (P213, GU049362) has the NS5B sequence for the same new subtype that P212 and P245 belong to, making this verified subtype 4w. Despite being represented by a single nucleotide that varies from all other genotype 4 sequences by more than 17.5%, isolate P026 is presently unassigned.

Similar to this, isolates KM45 and KM41 (DQ278891,3) have been identified as subtype 6 k [58]. However, they vary from each other and isolate VN405 (D84264) by 6.7 and > 17%, respectively, in the entire coding area sequence, leaving them to be a genotype 6 undefined subtype. Subtype 6u has been assigned to two different groups of isolates: EU408330-2 [51] and EU246940 [47]. The latter was submitted to

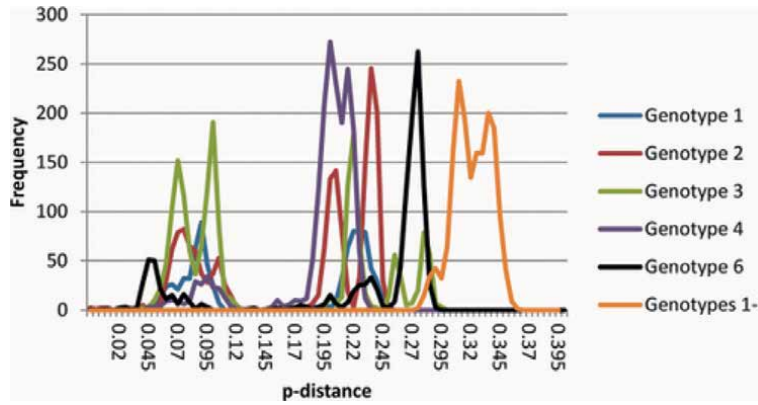

Figure 2.
Distribution of p-distances between sequences with entire coding regions. Using SSE, the frequency of p-distances within and between genotypes was determined. With the exception of subtypes 1a, 1b, and 2b, where 20 random sequences were employed [66], intra-genotypes pairwise distances were determined for all accessible full coding area sequences. Frequencies were adjusted to get the maximum frequency down to under 300 for p-distances >0.15 (corresponding to a percent difference of 15%). The frequencies scaled as above, and distances between genotypes were determined using one or two samples of each confirmed and unassigned subtype (adapted from [1]).

GenBank first and is represented by NS5B sequences from two additional isolates; as a result, it was given subtype 6u, whereas EU408330, EU408331, and EU408332 were given the subtype 6xa designation.

Finally, Smith et al. [1] analysis of sequence divergence and phylogenetic groupings opines that several isolates [52] classified as "subtype k-related" (TV257, KM35, QC273, TV476), "subtype l-related" (L349, TV533), "intermediate between subtypes 6m and 6n" (DH027), or "intermediate among subtypes 6j and 6i" (QC271) in their GenBank accessions should be regarded as unassigned novel subtypes.

2.1 Further levels of taxonomy

There are challenges in imposing a discrete categorisation method on a complicated taxonomy when defining this taxonomic difference between viral genotypes and subtypes. There are probably many taxonomic hierarchies, particularly for genotypes 3 and 6. For instance, a clade formed by many genotype 6 isolates with subtypes 6 k and 6 l [52]. These sequences and subtypes 6 m and 6n are part of a higher-level clade, while these subtypes and subtypes 6i and 6j are part of another grouping (**Figure 1**). The discontinuous distribution of p-distances across full coding area sequences (**Figure 2**), which consists of three practically overlapping ranges (approximately 15–20%, 20–25%, and 25–30%), reflects these evolutionary hierarchies.

2.2 Anticipated developments

The approach for categorising variations into genotypes and subtypes has proven unexpectedly reliable despite the increased amount and diversity of HCV sequences. The partitioning of the seven verified genotypes into subtypes that differ across a whole coding area sequence by >15% represents a natural break in the distribution of sequence distances, and the seven confirmed genotypes show significant bootstrap support (**Figure 2**). There are still some questions regarding the endemic region of genotype 5, which is represented by a single subtype that has been isolated in Europe, Brazil, North Africa, and South Africa, and genotype 7, which has been isolated from a Congolese immigrant. We may also expect to find more HCV-like viruses in the genus *Hepacivirus* [70–73], as well as variations that are more genetically related to HCV than the non-primate *Hepacivirus* that appears to be an endemic infection of horses globally [70].

3. Conclusion

This chapter established that there are 8 genotypes of HCV, and 93 subtypes. Of the 8 genotypes, 7 were highlighted in this chapter. The chapter also attempted to link HCV genotypes to their endemicity. Utilising the phylogenetic classification of HCV can provide a greater insight into eradicating the virus by 2030.

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Chapter 3

Host versus Virus: The Genetics in HCV Infection Leading to Treatment

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Abstract

The spread of hepatitis C virus (HCV) infection is a worldwide crisis. Intricate host-viral interactions control the HCV infection's natural course and treatment response according to new research. The patient's HCV genotype is the best predictor of response to pegylated interferon plus ribavirin therapy. The most crucial viral factor in determining the efficacy of direct-acting antiviral therapy is the HCV genotype 1 subtype. In addition to baseline viral load and HCV genomic heterogeneity, these two factors are linked with the treatment response. In previous large genome-wide association studies, interferon3 gene polymorphisms have been shown to be linked with spontaneous clearance and treatment responsiveness. An inosine triphosphatase gene polymorphism has been shown to reduce the risk of anaemia and other side effects caused by the antiviral drug ribavirin. In HCV patients, a second genetic mutation in the three-gene patatin-like phospholipase domain is associated with hepatic steatosis and fibrosis. This study examined the effects of viral and host genetics on the course and results of HCV therapy while concentrating on the known viral and host variables linked to HCV patient outcomes. This will result in fresh concepts for individualising both preventative care and therapeutic treatment.

Keywords: HCV infection, HCV host interaction, chronic hepatitis, CD8+ and CD4+ T-cell, IFN, progenitor cells

1. Introduction

Hepatitis C virus infection (HCV) is the main cause of chronic hepatitis, which is estimated to impact 70 million individuals globally. Cirrhosis, hepatocellular carcinoma (HCC), and end-stage liver disease may all result from chronic HCV infection. After their first HCV exposure, only 20% of individuals spontaneously clear the virus; chronic hepatitis is often the result [1]. Adaptive immune responses are expected to have a significant role in the progression of HCV infection. Indeed, during spontaneous viral elimination, robust and broadly directed CD8+ and CD4+ T cell responses that are specific to the virus are produced and remain following HCV clearance [2]. Furthermore, there is mounting evidence that neutralising antibodies (nAb) may help in viral eradication. The emergence of multiple unique but closely similar viral variants is one strategy used by HCV to escape the adaptive immune response.

Due to the complex population of HCV that circulates in vivo and is referred to be a "quasi-species" as a result of the high RNA-dependent RNA polymerase error rate and fast replication rate [3]. Adaptive immune responses favour the selection of persistent variant viruses that encode altered epitopes that, in this context, are either not recognised by T cells or antibodies at all or are only partly recognised by them. Additionally, several studies over the last 10 years have focussed on the functions of host genetic traits, such as favourable genotypes or alleles that may be a significant indication of clearance or be connected to a greater likelihood of sustained virological response (SVR) in treated patients [4]. In this chapter, we provide a summary of the knowledge about the role of host genetic factors in determining the course of HCV infection, as well as the role of antibodies and T cells in promoting in vivo HCV evolution [5].

2. Hepatitis C virus: an important virus for virology

In 1989, a virus that causes hepatitis (HCV) was first identified. In this year, conventional virology will give way to contemporary virology, which combines molecular science and biotechnology to research, characterise, and keep track of viruses. Traditional virology relied on the separation, growth, and biochemical study of viruses. HCV was the first infectious pathogen to be researched owing to molecular techniques, which are widely used to describe molecular components of HCV biology due to the virus' difficulty in replicating in vitro. Nowadays, HCV is rewriting history. Direct-acting antivirals (DAAs), which totally remove infection in more than 90% of patients, have challenged the notion that antivirals can only inhibit viral reproduction and slow disease processes. IFN-free DAA regimens are quickly taking the place of poorly tolerated IFN-based treatment regimens, stabilising, and perhaps even reversing tissue degeneration in patients with severe disease [6]. The creation of host-directed antiviral medicines has also been aided by thorough research into how host factors influence HCV. This chapter provides information on various important elements of viral behaviour at the cellular and host phases with an emphasis on the fundamentals and also most recent clinical developments crucial in assessing susceptibility to DAAs. Additionally, it offers illustrations of modern methods for genotyping and tracking viral replication.

3. HCV: life cycle and in vitro interactions between hosts and cells

Hepacivirus, Flavivirus, Pestivirus, and Pegivirus genera make up the huge group of encapsulated, single-stranded RNA viruses described as the Flaviviridae, which includes HCV [7]. This family of viruses, that includes several viruses spread via arthropods, is a developing public health affair [8]. Our understanding of numerous molecular pathways has been hampered by the difficulties in developing the in vitro replication model and the large network of cell surface particles that facilitate virus replication, which has contributed to poor understanding of the life cycle of HCV. While moving through the bloodstream, the HCV virion can either be a free particle or be held in check by host low-density lipoproteins [9]. It then adheres to the specific cell membrane and undergoes clathrin-mediated endocytosis to enter the cell. The viral genome, a 9.6 kb single-stranded RNA having positive charge, is exposed into the cytoplasm during endocytic division when the viral capsid is broken [10]. The

RNA genome directly translates into a single polyprotein precursor with about 3000 amino acid residues at the rough endoplasmic reticulum (ER). The precursor is then broken down into 10 mature products by cellular and viral proteases. Following a Golgi-dependent secretory pathway, new virions are accumulated in ER-derived compartments and cleared via exocytosis [11]. At this point, the virus has reached maturity and is encased in endogenous lipoproteins, that, as we will discuss below, is thought to aid immune escape. HCV virions have a reduced buoyant density and a broad size range because of their adhesion to host lipoproteins and also the absence of readily recognisable surface characteristics (40–80 nm diameter) [12]. In addition to supporting immune evasion, virus persistence, and DAA resistance, this process may also make it more challenging to pinpoint the cellular receptors required for viral entry. Additionally, it appears that the kind of cell affects the use of receptors as well as infection by free particles or cell to cell transmission [13]. HCV trans-complemented particles and cell culture-derived HCV (HCVcc) are two of the most often employed approaches for examining viral replication in vitro (HCVTCP). HCV genotype 2a variant JFH1, which has been obtained from a Japanese person having fulminant hepatitis, is used to reproduce HCVcc in Huh-7, a human cell line originating from hepatocellular cancer [14]. HCVcc produces infectious viruses, making it possible to characterise the shape and biochemical properties of virion particles, identify some HCV entry factors, and assess the effectiveness of DAA using native or inter-genotype transgenic JFH1 variations. Pseudotyped HCV virions are created by packaging transfected cells with viral proteins provided by various designs for HCVTCP, that have been fully detailed elsewhere [15]. Although HCVTCP can potentially be acquired from any isolate, it can only be infected once and cannot spread. Despite generally being thought to facilitate HCV replication, Huh-7 cells vary from native hepatocytes in regards to restriction mechanisms, HCV receptor localisation, and the absence of cell polarity seen in hepatic tissue [16]. Nearly all in vitro approaches, including HCVTCP and HCVcc, make use of Huh-7 cells. As a result, in vitro replication of viral entrance, organisation, discharge, and cell-to-cell dispersion is incomplete. HCV can replicate well in hepG2 cell clones and hepatoma cells created from primary hepatocytes, which may help the researchers understand better how virus interacts with its host cells [17].

4. Natural history of infection

Annual bacterial instances of up to 4 million cases are possible, while 130–200 million people worldwide are thought to have chronic HCV infections. Many of these afflicted people do not know whether they are infected or not. HCV mostly spreads through percutaneous contact with infected blood [18]. Many people acquired HCV before receiving a diagnosis as a result of poor injection techniques, infected blood, or infected blood products. In many developed countries, those who use injections or intranasal medications today suffer from the majority of infectious infections [19]. Particularly noteworthy, one of those often at risk of sexual transmission is HIV-positive men who participate in male-on-male sexual activity. Vertical transmission, transmission through piercings or tattoos, and other strategies are other strategies induced hepatic fibrosis and swelling may appear progressively over time. Cirrhosis, advanced liver disease, and HCC are long-term consequences [20, 21]. According to a large meta-analysis, over 20 years of infection, the likelihood of having cirrhosis rose from 7 to 18–41%. Men, ageing, drinking alcohol, and HIV co-infection are risk

factors for fibrosis, cirrhosis, and HCC [22]. Although 15–30% of infected people may, the majority do not display symptoms during acute disease. It is challenging to anticipate how many people with acute HCV infections will be cured of the disease without therapy because the majority of these infections are subclinical. Between 20 and 50% of individuals are thought to have spontaneous clearance within the initial 6 months of exposure; this is impacted by genetic factors, race, age, sexual orientation, and chronic diseases like HIV [23].

5. HCV and host interaction

The lipid-centric HCV virus can enter suitable host cells thanks to its 2 envelope glycoproteins, E2 and E1. To enter the cell, the 2 glycoproteins link with CD81 and the other external membrane proteins that are occludin, claudin-1, and the epidermal growth factor receptor. Clathrin-mediated endocytosis, where the nucleocapsid is expelled into the cytoplasm, is required for the virus to replicate within the target cell [24]. The host's immune system has access to the HCV genome after nucleocapsid is liberated into the cytoplasm. HCV proteins are translated via an internal ribosome binding domain that detects positive-strand RNA (IRES). During ER-related processing, HCV transforms a large polyprotein into structural and nonstructural proteins [25]. This is done by cellular and viral proteases. HCV RNA is unwound and stabilised in a reintegration complex with the help of NS5B and NS3 helicase regions, which control HCV replication. The development of "membranous web" structures, which serve as HCV replication chambers, is aided by NS4B [26]. Numerous host factors also promote HCV replication, such as Cyclophilin A, which interfaces with NS5A and NS5B to stimulate multiplication, and microRNA-122, which attaches to IRES to improve translation efficiency [27]. For assembly and release, HCV also makes advantage of fatty acid pathways and the creation of extremely low-density lipoprotein (VLDL). The HCV life cycle is shown in **Figure 1** with an emphasis on the critical phases of Virus replication, such as HCV adherence and entry into the host organism, HCV RNA reproduction, viral assembling and release, and HCV RNA translation to generate a giant polyprotein that is translated into 10 HCV proteins [28].

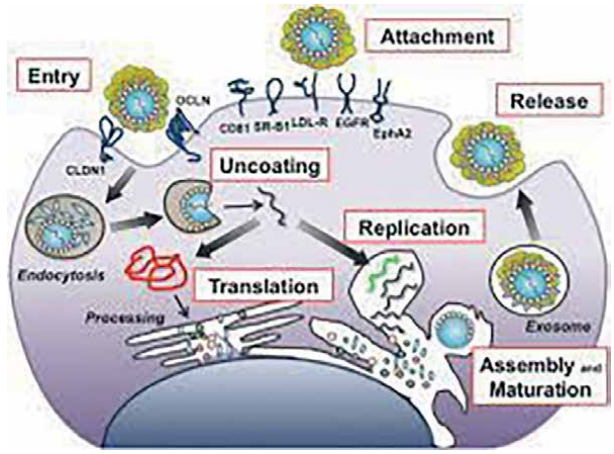

Figure 1.
Replication of hepatitis C virus inside the host cell [29]. (Licence Number: 5445310176889).

6. Host response and outcomes of the HCV infection

6.1 Innate immunity

When cells within the liver produce a variety of IFN-stimulated genes (ISGs), which restrict HCV replication and dissemination, the rate of rise drastically lowers. HCV has an exponential "ramp-up" stage of replication quickly after being infected in hepatic foci [30]. Adaptive immunity is sparked by innate immunity, which guards against HCV infection. The mitochondrial antiviral signalling (MAVS) proteins are induced by an interaction between the HCV RNA and the retinoic acid-inducible gene I [31]. Interferon-gamma (IFN-γ) production is started when dual RNA attaches to the Toll-like receptor-3 and signals through an adapter that contains a TIR domain (TRIF). Both procedures aid in the movement of IRF3 and NFB into the nucleus [32]. They encourage the production of pro-inflammatory cytokines and proinflammatory cytokines to mobilise and excite immune cells, as well as IFN and ISG to inhibit viral replication HCV's NS3-4A protease targets MAVS and TRIF to penetrate them, preventing the induction of IFN. Innate antiviral defences may be affected by HCV in a number of different ways [33]. ISG expression shows that even in HCV-infected hepatocytes with MAVS breakdown, these pathways do not completely suppress innate immunity. After HCV infection, hepatocytes selectively express IFN-γ. Progenitor cells, granulocytes, and other nonparenchymal cells can recognise viral molecular sequences and participate in IFN and cytokine secretion and response without increasing HCV replication [34].

6.1.1 The function of adaptive immune system in the development of infection

Innate antiviral responses rarely aid in the complete eradication of infection, despite the fact that they can restrict HCV multiplication and transmission in the absence of the host's natural defensive mechanism. Blood transaminase levels rise as viral loads decrease, a sign of hepatocyte cell death. An infection is deemed chronic when it persists for more than 6 months. Adaptive immunity initiates a cell-mediated reaction that targets several HCV epitopes and generates strong, broadly reactive neutralising antibodies to end infections (bNAbs) [35]. To lessen the chance of viral immunological escape, T cells concentrate on a variety of epitopes. HCV's ineffective reproductive strategy promotes quick evolution, immunological responses, and the selection of undetectable variations. Certain immunological escape mutations are undesirable because they decrease the virus's survival [36]. A second indication of successful anti-HCV immunity is the maintenance of polyfunctional T-lymphocyte activity. Whatever the outcome, HCV-selective CD4+ T lymphocytes are necessary for CD8+ T lymphocytes (and other immune cells) to function. By encouraging CD8+ T-lymphocyte survival, multiplication, and antiviral activity, HCV-specific CD4+ T-lymphocytes contribute to the healing of infection. To halt viral replication, effector T-lymphocytes assemble in the liver wherein they release the cytokines IFN and TNF, kill infected cells, and destroy infected tissues [37]. Key viral neutralising targets or stable domains necessary for hepatocyte infection are concealed by glycans, lipoproteins, and non-conserved decoy domains. BNAbs bind the necessary domains in place of the decoys. BNAbs may aid in eradication because HCV must continuously infect various target cells to retain even an established infection. T-lymphocytes in recurring HCV infection may concentrate on a narrower spectrum of epitopes; an initially broad response typically narrows [38]. This is in contrast towards the

extensive and persistent T-lymphocyte responses documented in the remission of HCV infection. Thus, immunological escape requires less alteration of the viral code. Immune-mediated selection for variations that evade CD8+ T cell identification is typically seen in chronic HCV infection. When viral antigenic patterns are intact, HCV-specific T-lymphocyte responses are characterised by a gradual function loss. Unrelated to epitope escape, a mechanism prevents CD4+ T-lymphocyte responses [39]. Without support from CD4+ T cells, CD8+ T cells cease growing, show signs of tiredness, and lose their capacity for communication. Damage to liver tissue may result from infiltrating inflammatory T cells, that are not always HCV-specific. Not to mention, individuals with latent infection have neutralising antibodies, though they may not manifest straight away and might be isolate-specific, often target hypervariable epitopes with increasing immunological escape potential [40].

6.1.2 The influence of the host's genetics on the infection's results

IFN-stimulated genes are linked to the collapse of IFN-based antiviral therapy, and individuals with these alleles are more probable to still have HCV infection. Increased ISG activity in the affected liver also suggests a bad outlook for IFN-based HCV therapy [41]. The frame-shifted IFN-4 gene in the IFN-β locus polymorphisms group suppresses the expression of IFN-4 protein as shown in **Figure 2**. IFN-4 may influence hepatocyte IFN responsiveness through negative feedback mechanisms, according to a theory [42]. IFN-4, on the other hand, may promote ongoing innate immune activation and impede the development of adaptive immunological responses. It is unclear if IFN-4 controls only innate immunity or also influences adaptive immunity. At the HLA locus, there are further significant polymorphisms [43].

6.1.3 Implications of the HVC diversity in transmission and pathogenesis

The onset of disease and viral evolution have been linked in studies utilising serially sampled HCV sequences. Cirrhosis, liver injury, and death can result from CHC, which can develop gradually and dependably or quickly. The results of the

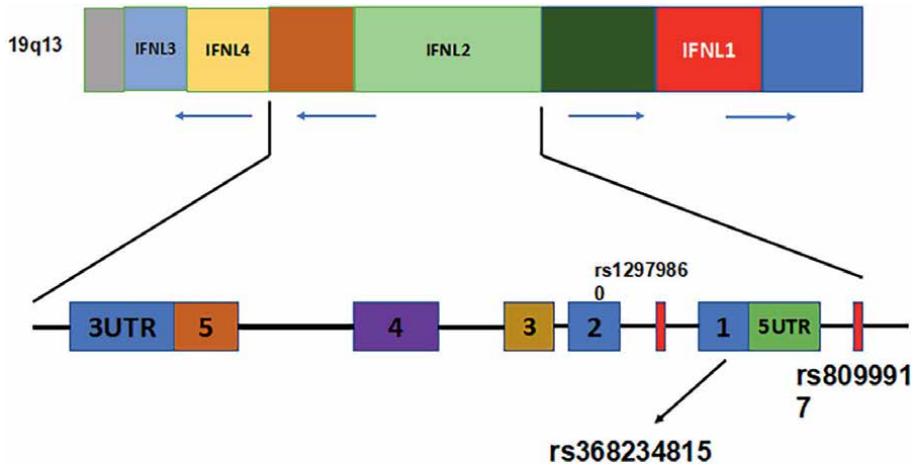

Figure 2.
The interferon lamda 3–4 (IFNL) gene, IFN 1, and two loci on chromosome 19q13 are represented, and these regions include important single nucleotide polymorphisms (SNPs) implicated in the elimination of HCV.

analysis of serial prospective study samples from hepatitis C patients associated with transfusions revealed a correlation between rapid illness progression and larger viral quasispecies variety and divergence, as well as higher percentage of synonymous substitution [44]. The mean substitution rates across all investigated viral covering sections were higher in instances with rapid infection progression over the course of the first 7 years of infection than in those with slower progression. Particularly for synonymous replacements, occurrences that advanced more quickly had a higher mean total number of changes per site [45]. These findings suggest that shorter viral generation times are associated with rapid disease onset, much like HIV-1. By doing a phylogenetic analysis on the full HVR1 amino acid sequence from every individual at different times in time, 2 topology sequences based on the progression of the disease were identified. Sequences from different eras blended together to form a mostly monophyletic community after hepatitis cases were settled [46]. Progressive hepatitis episodes showed a tendency for grouping over time and consistently longer branch lengths than instances of acute clearing hepatitis. This early neutralising restriction unexpectedly predicted the slow clinical progression of CHC. Patients with CHC who have cirrhosis have a 1–5% annual risk of developing HCC and a 3–6% yearly risk of hepatic decompensation after cirrhosis, both of which could necessitate an orthotopic liver transplant or lead to liver related death [47]. Additionally, both inside and outside the HVR1, the serum of HCC patients had a greater genetic diversity. On the partition of liver viruses, there is still a lot of disagreement. There was no proof of intra-hepatic E1/E2 quasispecies fragmentation when liver transplant recipients with end-stage liver disease were examined [48]. HCV enters cells by a complex mechanism involving a number of host proteins. In the viral life cycle, entry is a critical stage that may affect the evolution and diversity of the virus over time. The findings showed that there were 1–37 or more viruses that were transmitted and caused productive clinical infections [49].

6.1.4 HCV therapy

It is more challenging to develop vaccines, antiviral medications, and host defence awareness because of the diversity of quasispecies. Despite immunological activity, HCV frequently succeeds in getting past the host defence and maintaining a prolonged infection. There is general agreement that those who have CHC have compromised and lost their adaptive immunity [50]. CHC individuals who get antiviral therapy for viremia clearing; these individuals are still prone to infection after treatment has ended. The potential for re-infections from the same HCV subtype shows how difficult it is to develop a preventive HCV vaccination. Viral escape mutations are most common in the first 6 months following infection and can alter up to 50% of the CD8+ T cell-targeted epitopes [51]. Viral escape mutations are additionally uncommon during CHC, which might mean that T cell-mediated selective factors are not existent at this time. The way that specific CD8+ T lymphocytes are affected by HCV genetic mutations in terms of their capacity to reproduce the virus is essential. When the selected immunological pressure is released, the virus' fitness declines, which results in balancing substitutions or their swift reversion [52]. The preserved virus genomic portions are frequently targeted when viruses associated with these HLAs are subjected to CD8+ T cell responses, and escaping mutations are not well tolerated. These conserved epitopes are the obvious targets for developing a potent HCV T cell-based vaccine. To create a potent HCV vaccine, the correlations of protective immunity to combat HCV variation must be clarified. Despite the challenges in developing

an HCV preventative vaccine, antiviral research has been successful in treating the illness [53]. The cure rate for CHC has increased to almost 95% when direct-acting antiviral (DAA) medication was introduced in 2014 [54].

An important development in the treatment of HCV infection was the creation of the NS5B polymerase inhibitor sofosbuvir (SOF). Early chain termination happens as a result of the integration of SOF into newly synthesised viral RNA. NS5B's conserved active area is the target of SOF, which is effective against all HCV genotypes and has a high resistance barrier. Although SOF tolerance is sometimes shown in vivo, tissue culture can produce it [55]. Those with the SOF resistance mutation have very little viral replication (NS5B at location S282T). After 12 weeks of treatment, SOF, pegylated interferon alfa-2a, and ribavirin produced an SVR rate of 90% in patients with genotypes 1 and 4 infection. Similar to this, 12 weeks of treatment with an oral SOF + ribavirin combination led to SVR rates of 95% and 82%, respectively, in people with genotypes 2 and 3, in both treatment-experienced and naive patients [56]. In 2014, SOF and the NS5A inhibitor ledipasvir (LDV) were approved as a once-daily co-formulation for the treatment of HCV genotype 1. This combination was designed to quickly stop viral replication and prevent the spread of resistant strains [57]. After the course of 12 weeks of treatment, the SOF/LDV combination achieved SVR rates of 94–99% both with and without ribavirin. For the treatment of HCV genotype 1, SOF and SMV received approval in 2014 [57]. The NS3/4A protease inhibitors paritaprevir, ombitasvir, dasabuvir, and non-nucleoside NS5B polymerase inhibitors, as well as other protease inhibitors, can all be considerably improved by the HIV-1 protease inhibitor ritonavir. Patients with severe liver disease and all HCV genotypes were included in the investigations. SVR rates of more than 95% were achieved with regimens needing only 8 weeks of treatment, which is significant [58]. Another important factor is that innovative DAAs are extremely effective in some populations, such as elderly people, IDUs, people with severe liver disease, chronic renal illness, hemoglobinopathies, and HIV-1/HCV coinfection. In order to give medications with a higher metabolic profile and a wider ability to inhibit various HCV genotypes and variants, new DAAs are being developed [59].

6.1.5 Host-HCV interaction's effects on treatment

Pegylated interferon and ribavirin were previously the two main treatments for HCV infection, and SVR was only reached in a very small percentage of those who received treatment. Alopecia, arthralgia, sleeplessness, pyrexia, headaches, myalgia, tinnitus, and depression were among the unfavourable side effects frequently reported by patients getting interferon-based therapy [59]. DAAs have a quicker duration of treatment and a greater SVR than interferons, in contrast to being lower toxic and more effective. IL-1 induces persistent stimulation of innate immune-mediated inflammation. Innate immune activation has been found to be inhibited by DAA medication by reducing IL-1 release and NF phosphorylation. Because of this, there is fewer inflammation, which also means that liver fibrosis and damage are reduced [60]. The chemokines CXCL10 and CXCL11, which attract innate immune cells, are expressed less when DAA is treated with medication. The effectiveness of NK cells has also been linked to DAA treatment. The balance of the innate immune system is restored by reversing the impaired innate immunity caused by reduced chemokine release and normalising NK cell function. ISGs (interferon stimulated genes) were shown to be higher at baseline in HCV patients who had undergone DAA treatment by Alao et al. [61]. This finding raises the possibility that innate immunity plays a role

in the elimination of HCV. It is crucial to understand that RIG-I and TLR3 signalling are obstructed by the HCV NS3/4A protease, which pierces the human proteins MAVS and TRIF. It is unclear whether the direct antiviral activity of NS3/4A protease inhibitors or their capacity to activate the innate immune system's defence against viruses by preventing TRIF and MAVS hydrolysis is what ultimately rids the body of the virus. A viral infection is likely to spontaneously clear if a powerful early humoral immune reaction is developed via neutralising antibodies as during initial stages of an HCV infection. In HCV-infected individuals, early and substantial neutralising antibody development is connected with acquired immunity against viral persistence. It has also been demonstrated that the spontaneous clearance of acute HCV induces memory T-cell-driven preventative immunity [62]. This protective response is partially effective, but it is unable to protect reinfection with HCV strains that did not stimulate pre-existing memory T cells. Even though research on HCV vaccines is in various phases, none have achieved FDA approval. According to studies by Law et al., a singular oral HCV vaccine produced extensive attempt to cross antibodies against certain HCV genotypes [63]. Additionally, T-cell-mediated reactions were evoked. A human prophylactic T-cell-based HCV immunisation promoted the formation of both CD4+ and CD8+ T cells, according to the study by Swadling et al. The four following factors increase the likelihood of HCV infection: HCV has four main characteristics: (1) a high error-prone mutation rate with the ability to evade selective pressures by neutralising antibodies and CD8+ T cells; (2) genomic variability with seven genetic variants and more than 65 subtypes that differ in nucleotide pattern; (3) a mutational rate happening in the variable region zone 1 of E2 with the potential for HVR 1 to prevent antibody conditional to E2; and (4) cell-to-cell transmission of HCV [64]. Because circulating HCV binds to plasma lipoprotein to form an infected hybrid lipoviral particle (LVP), that promotes viral continuation and infection by limiting protective antibody access to envelop glycoprotein, the development of an effective HCV vaccine is greatly hindered. More research and development are required to create effective and safe HCV vaccines that encourage the production of pass immunoglobulins that target epitopes that are retained all over HCV genotypes and are unconnected to HCV escape. This is due to a risk of re-infection following HCV therapy. Given the notable nucleotide sequence changes between genotypes, it should be efficient against a variety of HCV strains [65]. A cell-mediated immune response as well as cross-neutralising antibodies can be generated by an HCV vaccination, while humoral immunity produced by vaccine highly immunogenic is insufficient to protect against HCV infections.

6.1.6 HCV resistance in relation to directly acting antiviral drugs

The majority of current anti HCV therapies do not use IFN. IFN-free methods frequently combine various DAA subcategories that focus on NS3/4A, NS5A, and NS5B as shown in **Table 1**. Alternative classifications for NS5B polymerase inhibitors include nucleotide or non-nucleotide counterparts [66]. The patient's cirrhotic condition is one of the most important host & viral factors that are known to affect the responsiveness to anti HCV treatment. Individuals with HCV infection who have never taken the DAA may have mutation spectra with substitutions linked to resistance (RASs). Globally, there is a wide variation in the prevalence of RASs based upon that viral genotype and origin [67]. It is not improbable that these mutations could be selected and impact a patient's reaction to medication. Any patient with HCV infection should have their medication resistance profile reviewed before beginning

DAA class	DAA (directed genotypes)
NS5B polymerase inhibitors of nucleotide and nucleoside	Daclatasvir (3)
	Sofosbuvir (1–4)
	Pibrentasvir (1–6)
	Ombitasvir (1,4)
NS3/4A protease inhibitors (Pls)	Sunvepra (1,4)
	Glecaprevir (1–6)
	Grazoprevir (1,3,4)
	Voxilaprevir (1–6)
	Galexox (1)
NS5A inhibitors	Velpatasvir (1–6)
	Ombitasvir (1)
	Elbasvir (1,6)
Inhibitors of the non-nucleoside NS5B polymerase	Dasabuvir (1)

Table 1.
The four kinds of DAAs, which are the cornerstone of anti-HCV therapy and are used in various combinations.

treatment. The American Association for the Study of Liver Diseases (AASLD) is indeed the organisation that strongly advises testing for antiviral resistance in patients who have not responded to NS5A inhibitors (and also in some patients who are dependent on DAA treatment who are treatment-naive), especially for genotypes (GT) 1a and 3. A rising number of studies have discovered that DAAs are less effective in treating HCV patients with susceptible strains. After 8 weeks and 12 weeks following diagnosis with ledipasvir/sofosbuvir, correspondingly, in diagnosis and treatment and treatment-naive persons, well before NS5A RASs with a 100-fold greater degree of sensitivity to ledipasvir than wildtype virus led to poorer SVR rates [68]. Findings from 35 clinical studies done in 22 countries suggest that the baseline presence of NS5A mutations affects the efficacy of a ledipasvir/sofosbuvir combo in GT1-infected individuals. People with GT1a infection who have had treatment are particularly impacted by this outcome. Elbasvir, a recently approved NS5A inhibitor, and grazoprevir, a recently approved NS3/4A protease inhibitor, were used in clinical studies on HCV resistance to demonstrate that amino acid replacements in NS5A at positions Met28, Gln30, Leu31, and Tyr93 significantly reduced treatment effectiveness when the variable viruses were pervasive at baseline in GT1a-infected patient populations (rates of 70% vs. 98% SVR12 for patients with and without NS5A mutations). According to the current recommendations, the identification of drug sensitivity for therapeutic measures would almost certainly be followed by the customisation of antiviral therapy and a higher probability of success [69]. Because of their limited fitness effect, NS5A RASs are an indication of RASs that, once identified, continue to develop over time at a specific rate. Treatment, which now affects between 2 and 10% of those with HCV, is frequently (though not always) connected to RAS selection. The European Association for the Study of the Liver (EASL) has released numerous reference materials and clinical guidelines that include lists of RASs that offer reduced susceptibility to DAAs in people and in vitro [70]. RAS incidence is a situation that is developing and rapidly evolving when different DAAs and DAA combinations are licenced to treat HCV infections. This scenario is anticipated by quasispecies

dynamics. RASs in NS3/4A and NS5A are frequently employed in individuals whose antifungal medication with NS3/4A and NS5A inhibitors failed [71]. After therapeutic failings with NS5B inhibitor-containing regimens, RASs in NS5B are detected much less frequently. Important residues of amino acids that resist nearly all NS3/4A inhibitors include Gln80, Arg155, Ala156, and Asp168. Locations Met28, Gln30, Leu31, Pro58, and Tyr93 in NS5A commonly choose replacements. In either situation, there is not much resistance. The long-term persistence of NS5A enzyme inhibitor mutations following the failure of NS5A inhibitor treatment also affects the therapeutic significance of these polymorphisms. Despite the relatively high barrier to resistance, several NS5B mutations, especially Leu159, Ser282, Cys316, Leu320, and Val321, have been linked to sofosbuvir resistance [72]. It has been demonstrated that the region of NS5B between amino acids 314 and 565 is sensitive to the non-nucleoside counterpart dasabuvir. Regarding RAS mutations connected to DAA drug failure, we need hard data. The HCV Italian popular resistance Network described the RAS profiles obtained by population sequencing study of 200 virological failures. They found a variety of tolerance tendencies that vary depending on the virus genetic, DAA regimen, and research study population, with a greater RAS prevalence at failure than previously reported [73]. Sarrazin et al. thoroughly analysed RAS patterns in a group of 626 non-responder/breakthrough and relapser patients to 2322 DAA-naive patients with HCV GT1 to GT4 [74]. They belonged to the group studying European HCV resistance. They discovered that the medication combinations' target regions, subtypes, and genotypes had a wide range of difficult activities. R155K in GT1a and D168E/V in GT1b RASs were usually utilised in NS3 after the failure of simeprevir- and paritaprevir-based treatments. Between GT1a and GT1b, a unique resistance profile in NS5A was found. When daclatasvir, ledipasvir, and ombitasvir therapy failed, Q30H/R were seen in GT1a patients, but Y93H were commonly picked in GT1b practiced medicine with NS5A inhibitors [75]. After treatment with daclatasvir/sofosbuvir, Y93H was frequently observed in GT3a. Patients may be moved to a nucleotide analogue and a blocker against a protein that was not targeted in the original therapy if they do not respond to the existing formulations of sofosbuvir with an NS3 or NS5A inhibitor. In reducing HCV viral load in patients, the recently licenced DAA sofosbuvir/velpatasvir/voxilaprevir seems to be more efficient than prior DAA-based drugs like NS3 and NS5A inhibitors [76]. The barrier to resistance is too low without sofosbuvir for the glecaprevir/pibrentasvir combination to be taken into consideration for the retreatment of patients who failed NS5A inhibitors. The glecaprevir/pibrentasvir combination cannot be considered for the reoperation of patient groups who lost NS5A inhibition because the barrier to resistance is too low. Sofosbuvir should be added as well [77]. Triple (or quadruple, if ribavirin is included) or quadruple medication combinations, one for each target, are widely employed. This approach is based on the notion that genetic resistance is increased when several amino acid alterations are required to produce the resistant phenotype. However, the following needs to be emphasised: A small number of RASs have been labelled as drug-class RASs, meaning they develop resistance to numerous medications in the same class. Because of their limited fitness effect, some RASs, once identified, continue to grow over time at a specified rate, as shown by NS5A RASs. As a result, the effectiveness of re-treatment alternatives may be affected, even with enhanced antiviral combinations [78]. Scientist team has identified a unique HCV antibiotic resistance mechanism that differs from the conventional resistance based on real amino acid alterations, complicating the understanding of HCV resistance. This technique was discovered after an HCV community was subjected to IFN 100 times in

a clone cell culture without any treatment and shown partial sensitivity despite having not received the medicine. This discovery was made in respect to several anti-HCV medications, each of which has a unique mechanism of action for effectively combating the virus (telaprevir, daclatasvir, ribavirin, cyclosporine A) [79]. Strength exercise was also resisted when sofosbuvir levels were high. This data refutes the hypothesis that the observed decline in viral susceptibility to inhibitors is caused by changes in the receptor that mediates receptor susceptibility: No substitutions that could be categorised as providing resistance to one of the inhibitors used were found in the I UDS analysis; similar levels of opposition were seen when infections with high-fitness virus infections were carried out over a 1000-fold range of multiplicity of infection (MOI), indicating that progeny manufacturing did not rely on the presence of averse strains, which would be clearly lowered with a 1000-fold decrease in MOI; and studied into a probable in vivo parallel and the clinical implications of strength and conditioning medication resistance has shown studies that demonstrate a proportion of patients cannot endure therapy even if there is no proof that resistance substitutions have arisen in the intended target [80].

6.1.7 Tools for assessing drug resistance include ultra-deep sequencing (UDS) technology

A fundamental problem in the investigation of HCV resistance is the standardisation of techniques for analysing amino acid alterations. The ability to identify RASs using various antiviral resistant testing techniques varies. Population sequencing might be helpful if such virus quasispecies were forced to keep the mutation that confers antiviral resistance [81]. On the other hand, hand, the architecture of a viral group is determined by the collection of mutations that go beyond the consensus sequence. Currently, it is possible to examine the mutant spectrum makeup using molecular clones, Sanger sequencing, and highly delicate methods such UDS platforms. Now, in order to develop bioinformatic methods for the research of viral quasispecies, the following significant obstacles must be overcome. A biased representation of the mutant spectrum may result from subpopulations being amplified by insufficient oligonucleotide primers. To determine a reasonable cut-off level for mutant frequency, erroneous mutations discovered during the sequenced and amplification phases should be checked in testing phases with regular clones. Artefactual crossover can be decreased by altering the PCR settings. Previously, 10,000 reads of sequencing depth were used to determine a cut-off number of 1% mutation frequency [82]. On the question of whether a given RAS can be considered clinically significant above a specific threshold for mutant regularity, the literature is mixed. It has been suggested that if the cutoff is 15%, the existence of a RAS may be disregarded. We do not believe that, or any other snipped level has any theoretical or practical support, for a variety of reasons. The detection rate for a particular variation depends on the quantity of readings (covers) obtained, the detection technique, and the composition of the HCV mutant spectrum. The upgraded UDS can find a certain variant more frequently depending on the number of readings. Due to how quickly it diverged into many genotypes and subtypes, HCV has truly come to represent true unpredictability [83]. For this reason, it would be preferable to have deep sequenced data both during the time of therapeutic failure and prior to the introduction of a new medicine, particularly if a lot of time passed since the failure of the last treatment. This is due to the possibility of the mutant spectra changing in a matter of seconds.

6.1.8 PRRs that detect viruses innately

The network of PRRs and related signalling pathways, which cause the production of IFN and inflammatory genes, are a crucial part of the innate immune system. The germline-encoded receptors (PRRs) found in plants, worms, drosophila, and mammals have not changed during the history of evolution. PRRs are encoded inside the host organism's germline, in contrast to the immunoglobulin receptors of the adaptive immune system, which are created through somatic gene rearrangements. These PRRs detect conserved microbial characteristics to prevent infection [84]. The classes of viral-sensing PRRs that will be discussed in this study include inflammasomes, Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), C-type lectin receptors (CLRs), and DNA sensors as shown in **Table 2**. Although these receptors are capable of detecting a wide variety of microorganisms, microbial metabolites, and host-derived harm molecular patterns, we will focus on the role of these receptors in the detection of viruses (DAMPs). Given the significance of these defences in defending the host from viral infections, it is not surprising that viruses have developed a range of ways to circumvent these antiviral defences. This makes it possible for viruses to proliferate and spread illness. Therefore, the topic of our discussion will be the most recent advancements in viral immune escape techniques.

6.2 Adaptive immunity and DAAs treatment

The emergence of HCV-specific T cells that produce the memory T cell marker CD127 and the antiapoptotic molecule Bcl-2 is proof that cytotoxic T cell reaction is restored after a person's natural recovery from HCV infection [98]. Weiland et al. found that people with chronic HCV infection had persistent TCF1 + CD127 + PD-1+ HCV-specific T cells that displayed signs of exhaustion and memory, and that were present both before and after DAA treatment. This subgroup was elevated in one patient who survived and had HCV-specific CD8 T cells that were terminally exhausted [99]. These CD8 T cells were different from memory CD8 T cells, which surface during spontaneous resolution (CD127+ PD-1−). Aregay et al. investigated the possibility that the diminished CD8T cellular response to HCV could be restored by DAA treatment. After HCV cure, every one of the 40 patients who underwent DAA treatment still had diminished functioning in their worn-out HCV-specific CD8 T cells [100]. Following HCV treatment, they observed a consistent shift in the subset makeup of CD8 T cells across all cirrhotic patients. The researchers deduced that the seriousness of the liver fibrosis in these cases was correlated with the prevalence of CD8 T cells with hyperfunction. These findings imply that the decreased phenotype of CD8 T cell reactions specific to HCV is not restored by DAA-induced HCV therapy. CD4 helper T cells are essential for an effective HCV-specific CD8 T cell reaction and for the spontaneous clearance of infection. Intrahepatic regulatory CD4 T cells and CD4(+) CD25(+) FoxP3(+) T-reg cells are more prevalent in patients with chronic HCV infection and do not decrease after viral eradication with IFN- or DAA-based therapy. Langhans et al. looked at T-reg cells at 12 and 24 weeks before and after taking DAAs. They discovered that T-reg cells survive after viral eradication for a long time and that DAAs do not alter their activation status [101]. Activation of the B cell response is associated with B cell-related illnesses such as combined cryoglobulins in 40–60% of patients and CV in 10–15% of patients. Comarmond et al. looked at the effect of DAAs on the CV of 27 patients with HCV infection. They

TLR(Ligand)	Virus(genome)	Outcomes	Reference
TLR7 (ssRNA)	Rota virus (dsRNA)	Favours host	
	West Nile virus (ss(+)RNA	Harmful/protective	[85]
	Avian Influenza	Favours host	[85]
	Chikungunya virus	Harmful to host	[86]
	HIV	Damaging to host	[84, 87]
	HSV1	Favours host	[86]
	VSV	Favours host	[88]
	Enterovirus 71	Favours host	[87]
TLR3 (dsRNA)	Poliovirus	Favours host	[89]
	Huntaan virus	Favours host	[90]
	Coxasackievirus	Favours host	[91]
	Punta Toro virus	Damaging to host	[84]
	EMCV	Favours host	[89]
	Influenza	Damaging to host	[89]
	HCV	Favours host	[90]
TLR4 (virus coat protein)	Influenza	Damaging to host	[92]
	RSV	Favours host	[92]
	VSV	Favours host	[92]
TLR6 (virus coat protein)	Dengue virus	Harmful to host	[93]
	RSV	Favours host	
TLR9 (CpG DNA)	Murine gammaherpesvirus 68	Favours host	[94]
	MCMV	Favours host	[95]
	ECTV	Favours host	[94]
	HSV	Favours host	[96]
	Dengue virus	Favours host	[95]
TLR2 (virus coat protein)	HSV	Favours host	[92]
	RSV	Favours host	[93]
	HCV	Harmful to host	[93]
TLR8 (ssRNA)	West Nile virus	Harmful to host	[97]

Table 2.
TLR-virus interactions and their effects on the host and the virus.

discovered that DAAs for CV led to the recovery of peripheral B cellular responses in around 88.9% of patients [102]. 90.2% of patients who underwent DAA treatment for vasculitis had a full clinical reaction, which was associated with a virological response, according to the Saadoun et al.'s examination of the vasculitis remission in 41 patients [103].

6.2.1 Liver disease-related proliferative signalling caused by HCV

The genesis and development of liver problems are reportedly aided by a variety of cell signalling alterations brought on by HCV infection, either indirectly or directly as shown in **Figure 3**. As HCV evolved, it altered these pathways to favour replication and persistence, which had a significant effect on viral pathogenicity and liver disease [104]. EGFR signalling is encouraged by the activation of the TGF and EGF pathways. STAT3 route: Both directly and indirectly, via the stimulation of NS5A and EGFR, which promotes the production of ROS, the activity of the core protein activates STAT3. Additionally, HCV silences PTPRD and SOCS3, two rival STAT3 regulators, by utilising miR-135a-5p and miR-19a. The TGF-pathway the UPR, which encourages NF-B activity, as well as the centre proteins, which directly interact with SMAD3, are two mechanisms by which HCV activates the TGF pathway. Endoglin (CD105) expression brought on by HCV encourages angiogenesis transmission and TGF-pathway stimulation. The HCV core triggers HIF-1, which boosts VEGF synthesis. Similar to this, STAT3-dependent androgen receptor stimulation allows HCV to enhance VEGF expression.

6.2.2 Immune system's role in the progression of liver damage after HCV infection that has lasted a long time

HCV lacks a dormant stage through its life span and is widely characterised as non-cytopathic, despite reports of apoptosis induction. It consequently persistently obstructs the liver's capacity to preserve homeostasis, resulting in stress and inflammation. A proinflammatory environment is produced by non-parenchymal cells that have been triggered by innate immune responses, and this milieu plays a significant role in the progression of liver illness from fibrosis to cirrhosis and HCC [105]. HCV is mostly recognised by TLR3 and RIG-I since it is a prototypical positive-stranded RNA virus. Nevertheless, it has a number of defences to prevent innate immune identification and control the subsequent IFN response. HCV enhances TLR3 signalling in monocytes and macrophages during a persistent infection. This causes the inflammasome to activate without IFN induction and the release of proinflammatory cytokines such interleukins (IL) [106]. It appears that natural killing (NK) cells are activated, IL-18

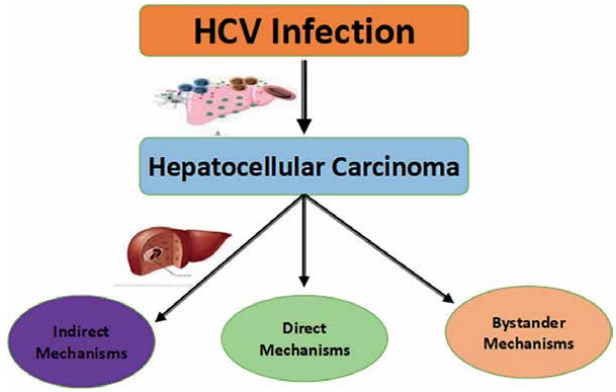

Figure 3.
Chronic HCV infection-related hepatocellular carcinoma processes include a mix of virus-mediated (direct), host-mediated (indirect), and host-related bystander effects.

is produced, and NLR3P inflammasomes are activated when macrophages recognise HCV-infected hepatocytes. The STAT3 signalling pathway is important during inflammation in complement to its pro-viral and proliferation effects. The signalling pathways STAT3 and NF-B are stimulated by the proinflammatory IL-6 and TNF, and when they are frequently activated, they can hasten the onset of liver problems and the emergence of HCC [107]. When JAK/STAT signalling is activated in neighbouring cells as a result of NF-B signalling, that also boosts IFN production, antiviral genes are produced. HCV proteins specifically target these actions while attenuating the innate antiviral response. Infected cells are also prevented from apoptosing by the HCV proteins core and NS5A, which do this by triggering NF-kB and AKT serine/threonine kinase (AKT).

Chronic HCV infection disturbs the equilibrium between both the ligand and the Fas receptor (FasR, CD95) (FasL, CD95L). It has been found that FasL-positive T lymphocytes interact with FasR-exposed hepatocytes to kill liver cells. FasR and FasL expression on hepatocytes and T cells, respectively, are strongly correlated with the severity of liver damage during chronic HCV infection [108]. Additionally, HCC exhibits nearly no FasR expression, which suggests a decreased susceptibility to T cell-mediated cytotoxicity and may increase the survival of tumour-genic cells. This result is also influenced by the absence of a functional T cell reaction during chronic infection. T cells, in particular CD8+ T cells, get exhausted after prolonged and continuous exposure to HCV antigens. Additionally, a lot of evidence points to the possibility of productive HCV infection of immune cells, particularly T cells, but it is unclear how much this can influence the immune response specifically directed against HCV [99]. According to the available data, myeloid-derived suppressor cells (MDSCs) in HCC patients and liver cancer animal models increase tumour growth and the incidence of metastases. HSCs are essential for both the control of the extracellular matrix of the liver and the healing of wounds.

6.2.3 Clinical implications of HCV-induced HCC risk biomarker identification

Just 30 years after discovery, HCV is now treatable thanks to the outstanding work of researchers, doctors, and the pharmaceutical industry. However, even in patients with severe liver disease, the viral cure brought on by treatment cannot entirely eliminate HCV-associated comorbid and HCC risk [109]. It is thought that epidemiologic peak of HCV-associated liver problems and HCC has not yet been reached because of the comparatively large time lag among virus infection liver damage and the emergence of HCC. This brings to light two important unmet medical needs for the clinical treatment of individuals with SVR: effective and secure chemopreventive programmes that support these individuals by tackling virus-specific pro-oncogenic mechanisms, epigenetic signatures, and liver fibrosis [110]. Viable biomarkers to estimate the proportion of SVR patients who are more likely to develop HCC. Finding a precise and comprehensive biomarker to assess the risk of HCC is difficult due to the relative range of individual aberrations. A 186-gene transcriptional profile that predicts HCC risk has been found in early-stage cirrhosis caused by HCV and non-tumour tissue close to HCC lesions [111]. Recent biomarkers, like the PES, that are based on virus-induced epigenetic modifications offer a novel perspective for determining the likelihood of HCC reappearance in HCV patients after SVR and make it possible to choose these people for clinical studies investigating HCC chemoprevention. Numerous extrahepatic side effects, including mixed cryoglobulinemia and B cell lymphomas, have been associated with chronic HCV infection. Though

the mechanisms underlying these problems have not yet been fully elucidated, it has been hypothesised that TGF and IL-6 play a part in their development [112]. For the HCC treatment that has already developed, a number of medicines that target HCV-relevant signalling pathways have also been suggested. In order to prevent and maybe treat HCC caused by other related liver cirrhosis aetiologies, such as NAFLD, new drug and individualised therapy strategies may be developed with the knowledge acquired from HCV-induced liver disease.

7. Conclusion

Since liver cirrhosis develops in one-third of people with persistent HCV infections, HCV is a prominent cause of liver disease. The acute hepatitis C may go away on its own or develop into chronic HCV infection, depending on the virus and host factors. To provide innate cellular immunity, NK cells release type II IFN and TNF, which stop viral multiplication by noncytolytic mechanisms, in addition to perforin and granzyme, which kill invading pathogens through cytolytic-dependent pathways. The adapted cellular approach to HCV infection, which eradicates the virus equally cytolytically and noncytolytically, depends heavily on CD8+ T cells. CD4+ T cells assist APC, B cells, and CD8+ T cells. Reduced capacity to control HCV infection is associated with cellular immunity failure. HCV escape mutation, reduced antigen presentation by HCV-infected DC, T cell exhaustion brought on by persistent HCV antigens, inadequate T cell primed by DC and intrahepatic antigen-presenting cells, and the formation of a tolerogenic intrahepatic milieu are all contributing factors to the persistence of HCV infection. Understanding how the host as well as the virus interact in respect of the variables that promote the settlement of the acute stage of a hepatitis C and the immunological evasive strategies the virus uses to sustain its survival in the host is critical. However, viral characteristics include HCV genetic variation, HCV cell-to-cell transmission, a rapid mutation rate, and the production of infectious lipoviral components hinder efforts to develop an HCV vaccine.

Conflict of interest

Authors declare no conflict of interest.

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Chapter 4

Multiscale Viral Dynamics Modeling of Hepatitis C Virus Infection Treated with Direct-Acting Antiviral Agents and Incorporating Immune System Response and Cell Proliferation

Hesham Elkaranshawy and Hossam Ezzat

Abstract

Mathematical models are formulated that describes the interaction between uninfected cells, infected cells, viruses, intracellular viral RNA, cytotoxic T-lymphocytes (CTLs), antibodies, and the hepatocyte proliferation of both uninfected and infected cells. The models used in this study incorporate certain biological connections that are believed to be crucial in understanding the interactions at play. By taking these relationships into account, we can draw logical conclusions with greater accuracy. This improves our ability to understand the origins of a disease, analyze clinical information, manage treatment plans, and identify new connections. These models can be applied to a variety of infectious diseases, such as human immunodeficiency virus (HIV), human papillomavirus (HPV), hepatitis B virus (HBV), hepatitis C virus (HCV), and Covid-19. An in-depth examination of the multiscale HCV model in relation to direct-acting antiviral agents is provided, but the findings can also be applied to other viruses.

Keywords: mathematical modeling, multiscale viral dynamics, hepatitis C virus HCV, immune system response, cell proliferation

1. Introduction

Hepatitis C virus (HCV) is a blood-borne virus that poses a significant threat to human health [1]. It can result in both acute and chronic hepatitis, with symptoms ranging from mild to severe lifelong illness. Many individuals with chronic HCV infection develop cirrhosis or liver cancer, with HCV being the leading cause of liver cancer. Globally, an estimated 71 million people have chronic HCV infection. The World Health Organization (WHO) estimates that 58 million people have HCV, with around 1.5 million new infections occurring annually, resulting in 290,000 deaths in

2019, primarily due to cirrhosis and liver cancer [2]. Treatment for chronic hepatitis C infection began in the 1990s with interferon-alfa, an injectable drug that improves the immune system rather than directly targeting the virus [3]. In 1998, the oral drug ribavirin (RBV) was added to interferon [4], and in 2002, pegylated interferon-alfa, a more durable and effective form of interferon, was approved [5]. Recent treatment options include direct-acting antiviral agents (DAAs) that target specific components of the HCV life cycle [6]. The rapid advancement in HCV drug development, with a cure rate of over 95% [2], has led to the prediction that full eradication of HCV may be possible in the absence of a vaccine. However, many obstacles still need to be overcome, including increasing awareness, improving access to care, developing, and making available simplified and highly effective drug regimens, increasing detection of infections, and securing funding for expertise [7, 8].

Mathematical modeling can be used to study and analyze engineering and physical problems, as well as biological processes such as heartbeats [9], neuron spiking [10], tumor growth and cancer treatment [11], and virus dynamics for viruses including HCV, HBV, HIV, and COVID-19 [12–14]. It is a valuable tool for understanding biological mechanisms and interpreting experimental results, predicting virus behavior under specific conditions, identifying parameters that promote disease spread, and estimating the number of medications needed to eradicate or control a disease [9]. Mathematical modeling is a valuable tool in the development of public health policies for controlling infectious diseases [15]. The early mathematical model for HCV was developed and analyzed as a system of ordinary differential equations (ODEs) which illustrate the fundamental dynamics of the virus within the body [16, 17]. Models for HCV treatment with DAAs therapy were also explored [18–20], and a new approximate analytical solution for solving the standard viral dynamic model for HCV has been proposed [21]. Stability analysis of basic virus models has been investigated [22].

When patients infected with HCV receive pegylated IFN or IFN in combination with ribavirin (RBV) as antiviral therapy, a biphasic decline in HCV RNA is typically observed. However, a triphasic decline has been reported in some patients [23–25]. Researchers have expanded on the basic original model [16, 17] by incorporating the proliferation of uninfected and infected liver cells to create a viral kinetic model with a triphasic pattern of HCV RNA decay [24–26]. Additionally, because the interactions between the replicating virus, liver cells, and various types of immune responses (such as cytotoxic T-lymphocytes (CTLs) and antibodies) are highly complex and nonlinear, mathematical models have been used to study these interactions and their stability [27, 28]. However, these models that account for liver cell proliferation and the immune system can only describe intercellular viral dynamics and not intracellular viral dynamics, which are necessary to understand the various antiviral effects associated with drug action mechanisms.

To address intracellular viral dynamics, researchers have developed mathematical multiscale models that account for the dynamics of intracellular viral replication and include the key stages of the HCV life cycle targeted by DAAs using systems of partial differential equations (PDEs) [29–33]. To overcome the limitations of numerical PDE solvers, Kitagawa et al. [34] proposed a new approach that transforms the standard PDE multiscale model of HCV infection into an equivalent system of ordinary differential equations (ODEs) without any assumptions. This transformed model eliminates the need for time-consuming calculations and is more widely available for mathematical analysis. Kitagawa et al. [35] also calculated the basic reproduction number of the transformed ODE model, investigated its global stability using Lyapunov-LaSalle's invariance principle, and studied all possible steady states of the model. Elkaranshawy

et al. [36] considered the local stability of this model using Routh-Hurwitz criterion and performed sensitivity analysis to determine the influence of each parameter on the basic reproduction number.

In this chapter, we review the progressive modeling of HCV kinetic, through presenting five models that show the gradual development of HCV mathematical models. The first model is the standard model of viral dynamics in the form of a system of ODEs. However, the model can only describe the intercellular viral dynamics. An approximate analytical solution is obtained for the standard model without simplification or reduction for the system of ODEs. The solution is used for the analysis of the model for patients treated with DAAs. It can also be useful for the estimation of parameters and for direct and simple predictions for the viral loads. The second model is a multiscale model in the form of a system of PDEs. The model can describe both the intercellular and the intracellular viral dynamics. The latter is required to capture the different antiviral effects corresponding to the action mechanisms of drugs. However, numerical PDE solvers are time-consuming and often converge poorly. Hence, a third model is introduced, which is the transformed ODEs multiscale model. In this model, the system of PDE in the previous multiscale model is converted into equivalent system of ODEs.

The cited models either incorporate hepatocyte proliferation and do not incorporate the dynamics of intracellular viral replication, or account for the dynamics of intracellular viral replication and do not account for the hepatocyte proliferation. The same observation can be made for incorporating the responses of immune system. Therefore, the fourth model presented in this chapter takes into consideration the hepatocyte proliferation of both uninfected and infected cells as well as both the intercellular and the intracellular viral dynamics. It is worth mentioning that both the classical PDEs multiscale model and the transformed ODEs multiscale model do not incorporate cell proliferation. From the point of view of mathematical analysis, considering cell proliferation with a multiscale model in the PDE form is an undesirable task. Hence, the fourth model is obtained by incorporating cell proliferation into the transformed ODEs multiscale model. The model represents a multiscale model for HCV treatment with DAAs therapy that can also clarify the observed HCV RNA triphasic viral decay and viral rebound to baseline values after the cessation of therapy. Numerical simulation and comparison with experimental data verifies the capabilities of the model to represent both the triphasic viral decay and viral rebound after cessation of therapy.

The fifth model in this chapter is composed to improve the realization of the interactions between HCV, drug treatments, infected cells, and immune system. The model takes into consideration the response of immune system as well as both the intercellular and the intracellular viral dynamics. Once again, it is worth mentioning that both the classical PDEs multiscale model and the transformed ODEs multiscale model do not incorporate immune system response. Considering immune system with a multiscale model in the PDE form is an undesirable task. Hence, the fifth model is obtained by incorporating immune system into the transformed ODEs multiscale model. Different antiviral effects of multidrug treatments are presented by defining three efficacies which are responsible for blocking intracellular viral production, blocking virion assembly/secretion, and enhancing the degradation rate of vRNA. Equilibrium points are determined, and stability analysis is presented. Stability analysis has shown the presence of five equilibrium points: one for an uninfected state, one for an infected state with no immune response, one for an infected state with a dominant antibody response but no CTL response, one for an infected state with a

dominant CTL response but no antibody response, and one for an infected state with a co-existing response from both CTLs and antibodies.

2. Standard HCV model

2.1 Mathematical model

Consider the system of nonlinear ODEs for the standard viral dynamic mathematical model for HCV kinetics during treatment [16–22]:

$$\frac{d\boldsymbol{T}}{dt} = s - d\,\boldsymbol{T} - \beta\,\boldsymbol{V}\,\boldsymbol{T} \tag{1}$$

$$\frac{d\boldsymbol{I}}{dt} = \beta\,\boldsymbol{V}\,\boldsymbol{T} - \delta\,\boldsymbol{I} \tag{2}$$

$$\frac{d\boldsymbol{V}}{dt} = (1-\varepsilon)\rho\,\boldsymbol{I} - c\,\boldsymbol{V} \tag{3}$$

where $\boldsymbol{T}$ are the target cells, produced at a constant rate $\boldsymbol{s}$ and died at a per capita rate $\boldsymbol{d}$. These cells can be infected by the virus, represented by $\boldsymbol{V}$, at a rate $\boldsymbol{\beta}$. Infected cells are assumed to die at a per capita rate $\boldsymbol{\delta}$. The model also includes the generation of virions at a rate $\boldsymbol{\rho}$ per infected cell and their clearance from the serum at a rate $\boldsymbol{c}$ per virion. The treatment is assumed to decrease the average viral production rate per infected cell from $\boldsymbol{\rho}$ to $(1-\varepsilon)\boldsymbol{\rho}$, where ε represents the in vivo antiviral effectiveness of the therapy $(0<\varepsilon<1)$. At the start of treatment $(t=0)$, the standard assumption is used, as stated in standard studies [9, 21, 22], that the system in the pretreatment state is in steady state, as given by:

$$\frac{d\boldsymbol{T}}{dt} = 0,\ \frac{d\boldsymbol{I}}{dt} = 0,\ \frac{d\boldsymbol{V}}{dt} = 0$$

Then,

$$T(0) = T_0 = \frac{c\delta}{\beta\rho}, I(0) = I_0 = \frac{-cd\delta + \beta\rho\mathrm{s}}{\beta\delta\rho}, V(0) = V_0 = \frac{-cd\delta + \beta\rho\mathrm{s}}{\beta c\delta} \tag{4}$$

2.2 Approximate analytical solution

Assuming a power series solution of order N to the three variables T, I, V in the following form:

$$T(t) = \sum_{i=0}^{N} T_i t^i \tag{5}$$

$$I(t) = \sum_{i=0}^{N} I_i t^i \tag{6}$$

$$V(t) = \sum_{i=0}^{N} V_i t^i \tag{7}$$

Substituting Eq. (5)–Eq. (7) into Eq. (1)–Eq. (3) and equating terms having the same powers of t. The c_{T_i}, c_{I_i}, and c_{V_i} coefficients can be calculated. For instant, the power series solution for $V(t)$ is

$$V(t) = \sum_{i=0}^{N} V_i t^i = V_0 + V_1 t + V_2 t^2 + V_3 t^3 + \ldots \tag{8}$$

where c_{V_0} is the initial condition of $V(t)$ and the coefficients c_{V_1}, c_{V_2}, and c_{V_3} are as follows:

$$V_1 = -\mathrm{c}\,\varepsilon\,V_0, V_2 = 0.5\,\mathrm{c}^2\,\varepsilon\,V_0, \tag{9}$$

$$V_3 = \frac{1}{6}V_0\left(-\mathrm{c}^3\varepsilon - \mathrm{c}^2\delta\varepsilon - \mathrm{c}\mathrm{d}\delta + \rho\mathrm{s} - \beta\mathrm{c}\delta V_0 + \varepsilon^2\mathrm{c}^2\delta + \mathrm{c}\mathrm{d}\delta\varepsilon - \rho\mathrm{s}\beta\varepsilon + \mathrm{c}\delta\beta\varepsilon V_0\right)$$

Using Laplace-Padé resummation method (PSLP) [21], the viral load $V(t)$ can be obtained as:

$$V(t) = A_1 e^{-D_1 t} + (V_0 - A_1)e^{-D_2 t} \tag{10}$$

where.

$$\mathrm{A}_1 = \frac{1}{2\sqrt{\mathrm{B}}}\left(V_0\sqrt{\mathrm{B}} + 3V_0V_1V_2 - 3{V_0}^2V_3 - {V_1}^3\right), D_1 = -\frac{A - \sqrt{B}}{f}, \tag{11}$$

$$D_2 = -\frac{A + \sqrt{B}}{f}, f = {V_1}^2 - 2V_2V_0, A = V_1V_2 - 3V_3V_0$$

$$B = 8V_0{V_2}^3 + 9{V_0}^2{V_3}^2 + 6{V_1}^3V_3 - 3{V_1}^2{V_2}^2 - 18V_0V_1V_2V_3$$

The general approximate analytical solution for the viral load for the standard dynamic model outlined in Eq. (1) through Eq. (3) can be represented by Eq. (9), Eq. (10), and Eq. (11). This solution considers the seven parameters present in the system of Eq. (1) through Eq. (3), accurately reflecting the biological characteristics inherent in the system. It should be noted that a simplified approximate solution, presented in [17], only includes three parameters.

2.3 Study cases

To demonstrate the capabilities and proficiency of the proposed method, four case studies are presented. These cases involve the examination of viral kinetics using the standard mathematical model of HCV dynamics. The PSLP method is utilized to solve the nonlinear dynamic model of viral kinetics for certain patients following the initiation of treatment with DAAs. In order to evaluate the performance of the PSLP method, the predictions are compared, for each patient, with viral load data found in literature sources [29, 30]. To obtain the best fit of data for each patient, parameters δ, ε, c, and ρ are estimated by utilizing Eq. (10) and the initial condition relation given in Eq. (4). The first case study examines patients who have been infected with HCV genotype 1 and treated with danoprevir. The viral load for these patients is monitored for 13 days after the initiation of danoprevir, and the data are available in [29]. The parameters are given in **Table 1**, and **Figure 1** demonstrates the comparison between the solution of PSLP method and the corresponding viral load data for each patient.

Patient	V_0 (IU/mL)	c (day)$^{-1}$	ε	δ (day)$^{-1}$	ρ (day)$^{-1}$
01-94GK	$10^{7.24}$	7.38	0.9995	0.15	149.684
03-94HD	$10^{6.72}$	12.44	0.998	0.29	151.188
03-94EA	$10^{5.79}$	10.5	0.998	0.17	11.212
03-94KG	$10^{6.98}$	9.4	0.98	0.35	246.748
04-94XD	$10^{6.63}$	10.26	0.9995	0.33	116.31

Table 1.
Parameter values used for the patients treated with danoprevir.

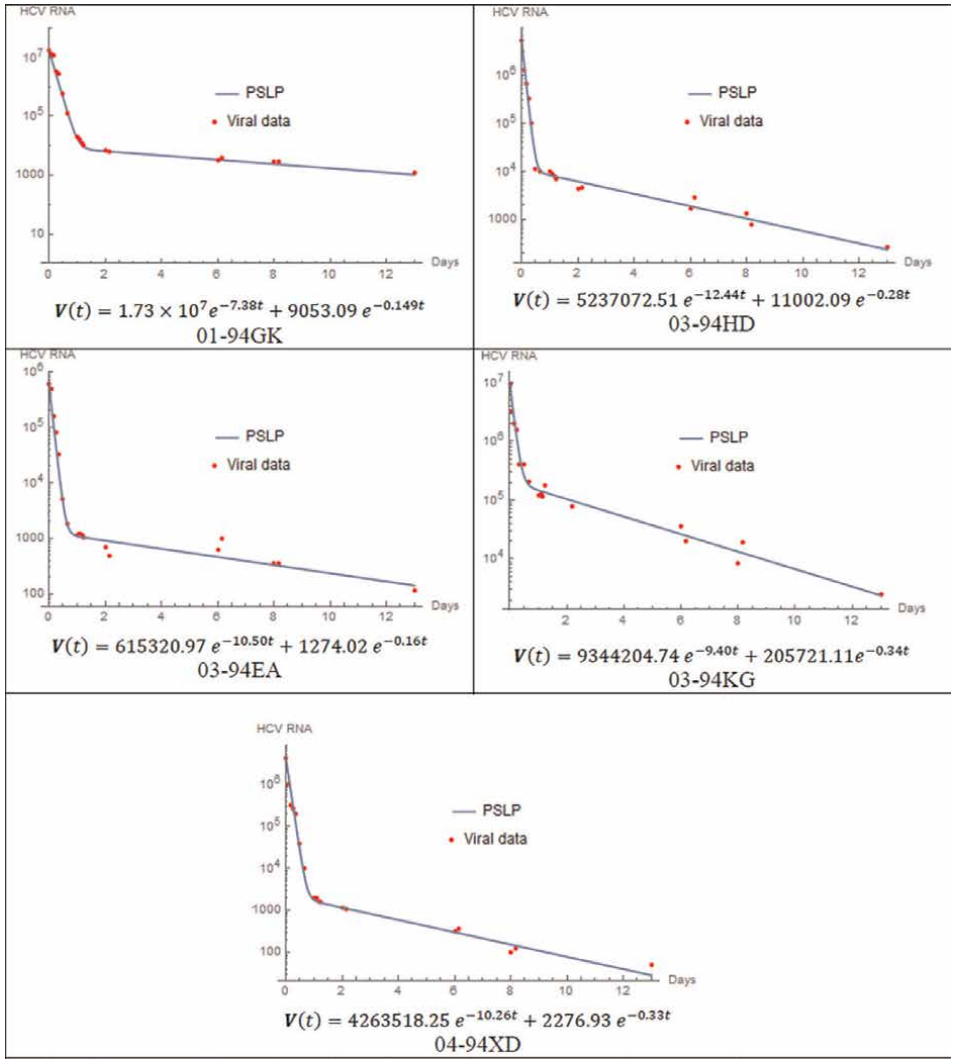

Figure 1.
Comparison between the approximate analytical solution using PSLP method and viral data for patients treated with danoprevir. $s = 1.3 * 10^5$*cells/ml,* $d = 0.01$ day^{-1}, $\beta = 5 * 10^{-8}$ *ml* day^{-1}virion^{-1}, *and the rest of parameter values are given in* ***Table 1***.

DAAs	c_{V_0} (IU/mL)	c (day)$^{-1}$	ε	δ (day)$^{-1}$	ρ (day)$^{-1}$
TVR + PR	$10^{5.98}$	5.28	0.999	0.27	8.18

Table 2.
Parameter values used with TVR + PR.

Another case study is presented, which examines an exploited combination of multi-drug DAAs treatments. The predictions of the PSLP method are compared with the corresponding viral load data obtained through simulation in [31]. The parameter values used in this case are listed in **Table 2**, with δ, ε, and c having the same values assigned in [31], and ρ chosen accordingly. **Figure 2** displays the PSLP results and the published simulation results for 25 days after the initiation of treatment with TVR + PR. The PSLP solution and the simulation results are highly similar. This comparison demonstrates that the proposed PSLP method can provide appropriate approximate analytical solutions for all considered cases. It is worth mentioning that in this case, the rate of cure is close to 100%.

The PSLP solution provides a valuable tool for medical specialists and physicians to predict a patient's response to a specific treatment regimen before the treatment begins. They can use the patient's parameters to calculate the constants in Eq. (9) and Eq. (11) and substitute them in Eq. (10) to obtain a closed-form solution for the viral load. This allows them to plot the viral load over time or estimate the viral load at any given point in time by direct substitution in the closed-form solution. The ability to

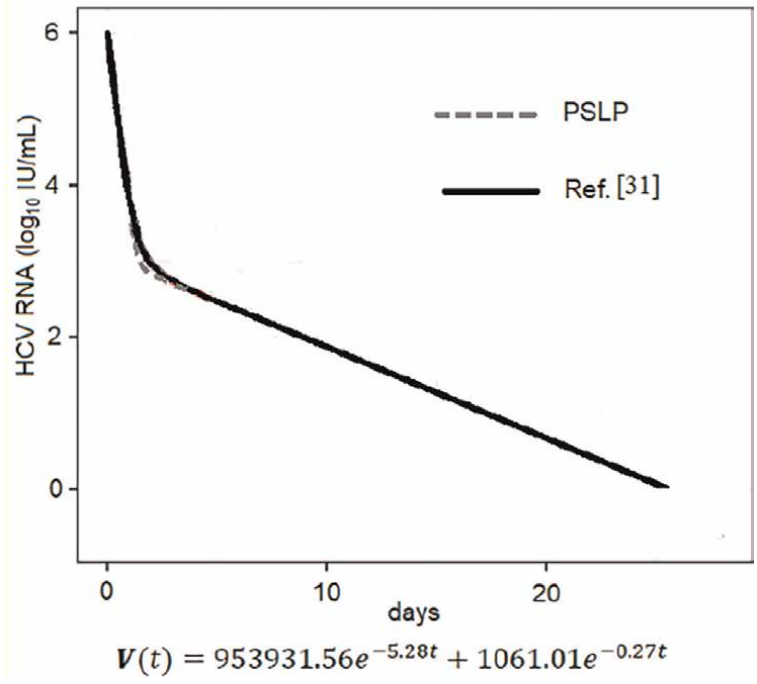

Figure 2.
*Comparison between results obtained by PSLP method and by simulation in [31] for treatment with TVR + PR. $s = 1.3 \times 10^5$cells/ml, $d = 0.01$ day^{-1}, $\beta = 5 \times 10^{-8}$ ml day^{-1}virion^{-1}. **Table 2** Gives the values for the rest of parameter.*

predict a patient's response to treatment before it starts is highly desirable for efficient and effective medical care.

3. Classical PDEs multiscale model

A multiscale model in the form of PDEs, which describes the intracellular life cycle had been proposed and applied by many researchers to analyze clinical data under multidrug treatment [29–33]. The model is as follows:

$$\frac{\partial R(a,t)}{\partial t} + \frac{\partial R(a,t)}{\partial a} = \alpha(1-\varepsilon_\alpha) - ((1-\varepsilon_s)\rho + k\mu)R(a,t) \tag{12}$$

$$\frac{dT(t)}{dt} = s - d\ T(t) - \beta\ V(t)T(t) \tag{13}$$

$$\frac{\partial i(a,t)}{\partial t} + \frac{\partial i(a,t)}{\partial a} = -\delta\ i(a,t) \tag{14}$$

$$\frac{dV(t)}{dt} = (1-\varepsilon_s)\rho \int_0^\infty R(a,t)i(a,t)\ da - c\ V(t) \tag{15}$$

with the following boundary conditions: $R(0,t) = \zeta$, $i(0,t) = \beta\ V(t)T(t)$. $R(a,t)$ is the age and time distribution of intracellular viral RNA (vRNA) in a cell with infection age a. The parameters α and μ represent the production and degradation rates of intracellular vRNA, respectively. It is assumed that vRNA assembles with viral proteins and is released from an infected cell as viral particles at a rate of ρ. The efficacies ε_α, ε_s, and k play a role in inhibiting intracellular viral production, preventing the assembly and/or secretion of virions, and increasing the degradation rate of vRNA, respectively. The entry virus-derived RNA starts to replicate from ζ copies in a newly infected cell.

4. Transformed ODEs multiscale model

Kitagawa et al. [34, 35] transformed the previous multiscale PDEs model into a mathematically alike ODEs model without any assumptions. The transformed model can be written as:

$$\frac{dT(t)}{dt} = s - d\ T(t) - \beta\ V(t)T(t) \tag{16}$$

$$\frac{dI(t)}{dt} = \beta\ V(t)T(t) - \delta\ I(t) \tag{17}$$

$$\frac{dP(t)}{dt} = \zeta\ \beta\ V(t)T(t) + \alpha(1-\varepsilon_\alpha)I(t) - (k\mu + \rho(1-\varepsilon_s) + \delta)P(t) \tag{18}$$

$$\frac{dV(t)}{dt} = \rho(1-\varepsilon_s)P(t) - c\ V(t) \tag{19}$$

where $I(t)$ denotes the total number of infected cells and defined as $I(t) = \int_0^\infty i(a,t)\ da$, and $P(t)$ is the total amount of intracellular vRNA pooled in all infected cells and defined as $P(t) = \int_0^\infty R(a,t)i(a,t)\ da$.

5. ODEs multiscale model incorporating cell proliferation

The transformed model can predict a biphasic decline for the viral load. It cannot predict a triphasic viral decay. To explain this, let us first assume the existence of a shoulder viral load and then prove that this is not possible for the model. For the shoulder, we can consider $V(t)$ as constant, that means that $\frac{dV(t)}{dt} = 0$, which can be substituted to Eq. (19) to get that $P(t)$ is also constant which means that $\frac{dP(t)}{dt} = 0$. Since $T(t)$ is increasing, Eq. (18) indicates that $I(t)$ is decreasing. If these are substituted in Eq. (17), it means that $\frac{dI(t)}{dt}$ is increasing. However, since $I(t)$ is always positive, pointing that $\frac{dI(t)}{dt}$ is increasing is in contradiction with $I(t)$ decreasing. Hence, the transformed model presented in (2) cannot predict the shoulder phase. It is worth mentioning that whatever is confirmed for the transformed model is also confirmed for the classical multiscale model presented by Eq. (12)–Eq. (15).

The transformed ODE model ignores proliferation of both infected and uninfected cells, though hepatocytes have been suggested to be the major producers of HCV. We assume that target cells are hepatocytes and suggested to include the density-dependent proliferation terms for both infected and uninfected hepatocytes that only allow growth of the liver until a maximum size, T_{max}, is reached.

5.1 Mathematical model

The extended model is given by:

$$\frac{dT(t)}{dt} = s - d\,T(t) - \beta\,V(t)T(t) + rT(t)\left(1 - \frac{T(t)+I(t)}{T_{max}}\right) \tag{20}$$

$$\frac{dI(t)}{dt} = \beta\,V(t)T(t) - \delta\,I(t) + rI(t)\left(1 - \frac{T(t)+I(t)}{T_{max}}\right) \tag{21}$$

$$\frac{dP(t)}{dt} = \zeta\,\beta\,V(t)T(t) + \alpha(1-\varepsilon_\alpha)I(t) + \zeta\,r\,I(t)\left(1 - \frac{T(t)+I(t)}{T_{max}}\right) - (k\mu + (1-\varepsilon_s)\rho + \delta)P(t) \tag{22}$$

$$\frac{dV(t)}{dt} = (1-\varepsilon_s)\rho P(t) - c\,V(t) \tag{23}$$

where uninfected $T(t)$ and infected $I(t)$ hepatocytes can proliferate with maximum proliferation rate r, under a blind homeostasis process, in which there is no distinction between infected and uninfected cells in the density-dependent term. The logistic terms describe the proliferation of the uninfected cells T and the proliferation of the infected cells I that are limited by the maximum size T_{max}. Hence, the saturation effects of both the uninfected and the infected cells are contained in the model. Therefore, the model could not predict unrealistic unlimited increases in the values of these cells. Also, it can be noticed that the proliferation terms assumes that the growth rate decreases linearly with the increase of the total hepatocytes population $T(t) + I(t)$ until it reaches zero at the maximum size.

By inclusion of proliferation of hepatocytes in the extended model, the model can predict viral kinetics in chronic HCV patients during and after antiviral therapy. The

shoulder phase, and hence a triphasic viral decay, occurs if the number of uninfected cells is much lower than the number of infected cells before therapy, and the rate of proliferation plus the rate of de novo of infected cells equals the rate of infected cell loss. As the number of uninfected cells increase, proliferation of infected cells slows and ultimately reaches a point at which the number of infected cells starts to decline. Hence, the third phase of viral decline starts.

Unlike the transformed model which predicts virus resurgence to pretreatment levels with damped oscillations after cessation of therapy, the extended model predicts virus resurgence to pretreatment levels without oscillations after cessation of therapy, as can be seen in the following sections. The kinetics of viral resurgence thus tends to mimic that observed in patients taken off therapy.

The model incorporating cell proliferation can predict both a biphasic decline and a triphasic decline. To prove this, we assume the existence of a shoulder viral load and then confirm that this is possible for the extended model. For the extended model, let $V(t)$ be constant at the shoulder which means that $\frac{dV(t)}{dt} = 0$ and substitute into the fourth equation in model (3) to get that $P(t)$ is also constant which means that $\frac{dP(t)}{dt} = 0$. For the third equation in this model, it is not a must that $I(t)$ is decreasing since $T(t)$ is increasing because the proliferation term is decreasing and can compensate for the increase in $T(t)$ even if $I(t)$ is constant. The same argument is applied for the second equation in model (3), and the increase in the first term can be compensated by the decrease in the proliferation term. Hence, for constant $I(t)$, the model (3) can predict $\frac{dI(t)}{dt} = 0$. Therefore, the model can predict the shoulder phase for the viral load.

5.2 Biphasic and triphasic viral decline

The results from the proposed model are compared with the published experimental data. Experimental HCV RNA data, for two patients treated with peginterferon α-2a alone [17, 24], and in combination with ribavirin [23, 25], are presented. The values assigned for the parameters for each patient are given in **Table 3**. The first patient exhibits a biphasic decline. **Figure 3**, for a patient treated with peginterferon α-2a alone, shows that the viral load has a phase of rapid decline at the beginning followed by a phase of normal decline. The second patient, treated with peginterferon α-2a in combination with ribavirin, exhibits triphasic decline. The triphasic decline consists of a first phase with rapid virus load decline, followed by a *shoulder phase* in which virus load decays slowly, and a third phase of renewed normal viral decay as can be seen in **Figure 4**. **Figures 3** and **4** show that the proposed model can present both the biphasic and the triphasic viral decline, and it can be noticed that the proposed model is consistent with the data.

5.3 Viral kinetics after treatment cessation

In most treated individuals, the virus returns to its pretreatment levels within 1–2 weeks after cessation of treatment [23]. To consider this phenomenon, the drug efficacies ε_α *and* ε_s are assigned at time 0 for 80 days, as given in **Table 4**, and then these efficacies are set to 0 for the rest of the simulation. **Figure 5** shows that with the inclusion of proliferation in the proposed model, the virus returns to its pretreatment level quickly.

Parameter	Patient 1	Patient 2	Units
T_{max}	0.7×10^7	0.6×10^7	cell ml^{-1}
s	8×10^5	0.62×10^3	cell ml^{-1} day^{-1}
β	0.6×10^{-7}	0.5×10^{-7}	ml day^{-1} $virion^{-1}$
d	4.7×10^{-3}	8.7×10^{-3}	day^{-1}
δ	0.3	0.24	day^{-1}
c	5.9	4.4	day^{-1}
r	0.45	0.5	day^{-1}
ρ	5.4	6.3	day^{-1}
ζ	1	1	*viron cell*$^{-1}$
α	14	21	*viron cell*$^{-1}$ day^{-1}
μ	1	1	day^{-1}
ε_s	0.906	0.899	
k	1	1	
ε_α	0.97	0.972	

Table 3.
Parameter values used in fitting model with HCV RNA data of patients.

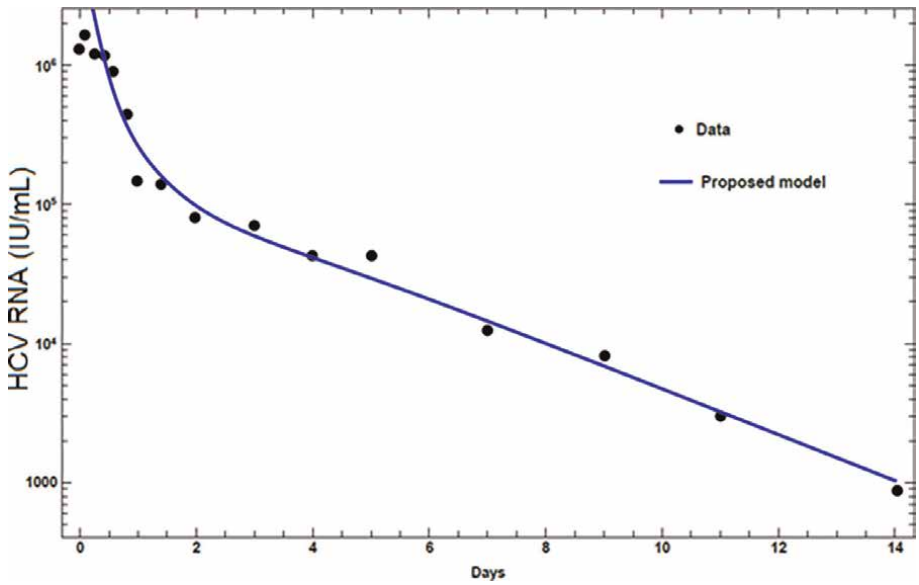

Figure 3.
Fitting viral loads from the model with experimental data for patient 1.

5.4 Discussion

The model explores the dynamics of HCV infection under therapy with DAAs including both the intracellular viral RNA replication/degradation and the

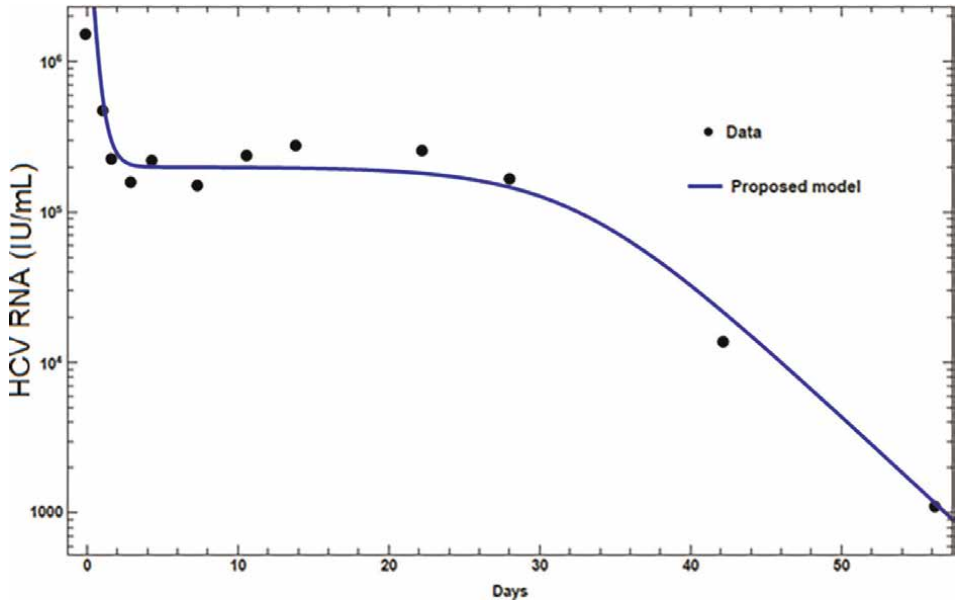

Figure 4.
Fitting viral loads from the model with experimental data for patient 2.

Parameter	Value and units	Parameter	Value and units
s	13×10^5 cell ml^{-1} day^{-1}	α	40 *viron cell*$^{-1}$ day^{-1}
β	5×10^{-8} ml day^{-1} virion^{-1}	μ	1 day^{-1}
d	0.1 day^{-1}	c	22.3 day^{-1}
T_{max}	1.35×10^7 cell ml^{-1}	ρ	8.18 day^{-1}
r	2.4 day^{-1}	k	1
δ	0.14 day^{-1}	ε_α	0.99
ζ	1 *viron cell*$^{-1}$	ε_s	0.56

Table 4.
Parameter values used in the numerical simulation.

extracellular viral infection with age dependency and time dependency. The model consists of a system of nonlinear ODEs instead of PDEs in classical multiscale model. Therefore, numerical computation is more efficient, time-saving, and convergent.

Numerical studies prove that the model can represent the triphasic patterns profile in HCV RNA decay which had been recorded for a class of patients. It can also represent the viral rebound to pretreatment levels after therapy cessation without oscillation. Moreover, agreement of the model with experimental data is confirmed.

6. ODE multiscale model incorporating body immune system

An extension to the transformed multiscale ODE model is presented. Two additional variables are included in the transformed model to account for the immune

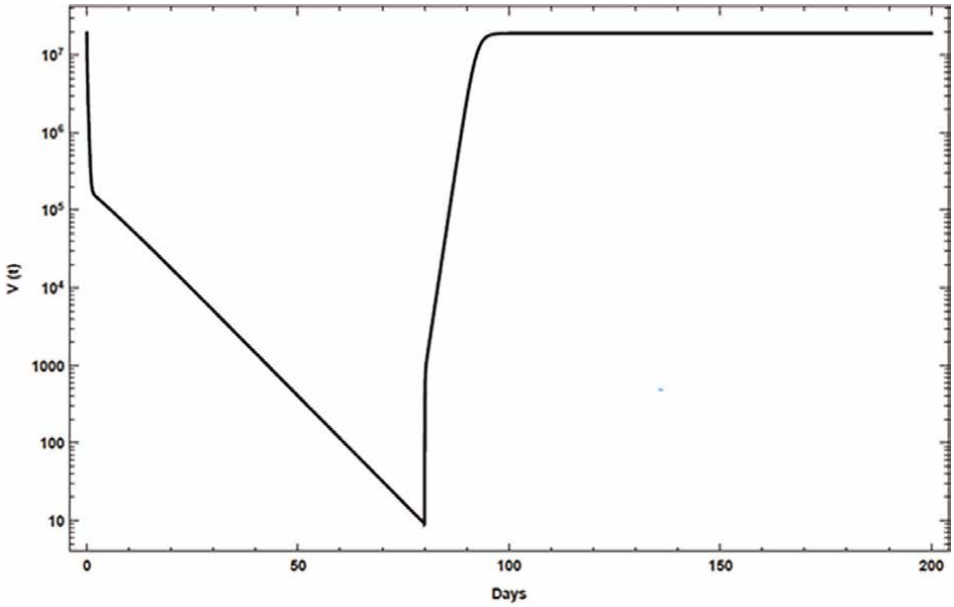

Figure 5.
Simulation of viral kinetics after therapy cessation using the model, case 1.

system response. The first variable, $Z(t)$, represents the number of CTLs, which are responsible for destroying infected cells and thereby inhibiting the reproduction of the virus. The second variable, $W(t)$, represents the number of antibodies generated, which play a role in neutralizing the virus in vivo.

6.1 Mathematical model

The new model is described by the following ODEs system:

$$\frac{dT(t)}{dt} = s - r\,T(t) - \beta\,V(t)T(t) \tag{24}$$

$$\frac{dI(t)}{dt} = \beta\,V(t)T(t) - \delta\,I(t) - f\,I(t)Z(t) \tag{25}$$

$$\frac{dP(t)}{dt} = \beta\,V(t)T(t) + \alpha(1-\varepsilon_\alpha)I(t) - (k\mu + (1-\varepsilon_s)\rho + \delta)P(t) \tag{26}$$

$$\frac{dV(t)}{dt} = \rho(1-\varepsilon_s)P(t) - c\,V(t) - q\,V(t)W(t) \tag{27}$$

$$\frac{dZ(t)}{dt} = u\,I(t)Z(t) - b\,Z(t) \tag{28}$$

$$\frac{dW(t)}{dt} = gV(t)W(t) - h\,W(t) \tag{29}$$

The term $f\,I(t)Z(t)$ in Eq. (25) represents the rate at which infected cells are killed by the CTL response, and the term $q\,V(t)W(t)$ in Eq. (27) represents the rate at which virus particles are neutralized by the antibodies. CTLs become activated in response to

viral antigens from infected cells and once activated, they divide and their population grows (clonal expansion). Therefore, in Eq. (28), CTLs increase at a rate of $uI(t)Z(t)$ and decay at a rate of $bZ(t)$ due to the lack of antigenic stimulation. Antibodies are produced by B cells and are initially attached to them, serving as receptors that can recognize the virus. When B cells are exposed to a free virus, they divide and secrete the antibodies. Thus, antibodies progress at a rate $gV(t)W(t)$ and decay at a rate $hW(t)$ in Eq. (29).

6.2 Basic reproduction number

The basic reproduction number, denoted as R_0, is defined as the expected total number of viral particles newly produced during the entire period of infection from one typical viral particle in a population consisting only of uninfected cells. It is calculated under no treatment conditions and is also computed at the disease-free equilibrium point E_0. No treatment can be specified by assigning $\varepsilon_\alpha = 0$, $\varepsilon_s = 0$, and $k = 1$ in the presented model, and E_0will be obtained in the following section. This basic reproduction number explains the average number of newly infected cells based on the dynamics of the total amount of intracellular viral RNA, which corresponds to $P(t)$ in the transformed ODE model, instead of the dynamics of the individual amount of intracellular viral RNA in the original PDE model. Note that the life cycles of both extracellular viral and total intracellular viral RNA are explicitly considered in the ODE model, and the viruses are formulated from the viral RNAs.

There are several methods that can be used to obtain the basic reproduction number, such as the next-generation method, which was introduced by Diekmann et al., [37]. There are two main approaches to apply this method, as explained by Driessche and Watmough [38] and by Castillo-Chavez, et al., [39]. In this work, the second approach is used, and proofs and further details can be found in [38–40]. The obtained form for R_0 is given by:

$$R_0 = \frac{\beta\, s\, \rho(\alpha + \delta)}{c\, r\, \delta(\mu + \rho + \delta)} \tag{30}$$

6.3 Equilibrium points

Equilibrium points are the values of the variables T_*, I_*, P_*, V_*, Z_*, and W_*, under no treatment, at which the derivatives of these variables, i.e., the left-hand sides in Eq. (24)–Eq. (29), vanish. These equilibrium points represent the steady state values of the variables after the cease of medication. In fact, in stability analysis, the interest is in specifying the behavior of the virus after the cease of medication. Commercial program *Mathematica 12 program* is used to solve these algebraic equations. The program gives six equilibrium points; however, one of them has negative coordinates that have no biological meaning. The five other points are as follows:

$$E_0 = \left(\frac{s}{r}, 0, 0, 0, 0, 0\right) \tag{31}$$

$$E_1 = \left(\frac{s}{r\, R_0}, \frac{s}{\delta}\left(1 - \frac{1}{R_0}\right), \frac{c\, r}{\beta\rho}(R_0 - 1), \frac{r}{\beta}(R_0 - 1), 0, 0\right) \tag{32}$$

$$E_2 = \left(k_2 s, \frac{\beta h \mathrm{S}}{\delta g} k_2, \frac{c\, r\, h R_0}{g\rho} k_2, \frac{h}{g}, 0, \frac{c}{q}(-1 + r\, k_2\, R_0)\right) \tag{33}$$

$$E_3 = \left(\frac{k_1 + 2c\,r\,u\,\mu_1 - k_3}{2\beta\,r\,\rho u}, \frac{b}{u}, \frac{k_1 + k_3}{2\beta\,\rho\,\mu_1 u}, \frac{k_1 + k_3}{2\beta\,c\,\mu_1 u}, \frac{(k_1 + k_3)}{2\,b\,\beta\rho f} - \frac{(\alpha + \delta\,)}{f}, 0\right) \quad (34)$$

$$E_4 = \left(k_2 s, \frac{b}{u}, \frac{c\delta\,r\,R_0}{s\rho(\alpha + \delta)}\left(\frac{\alpha\,b}{u\,\beta} + \frac{s\,k_2 h}{g}\right), \frac{h}{g}, \left(\frac{\beta\,h\,s\,u}{brgf + b\beta hf} - \frac{\delta}{f}\right), \left(\frac{\alpha\,bg\rho}{h\mu_1 qu} + \frac{\beta k_2 \rho s}{\mu_1 q} - \frac{c}{q}\right)\right) \quad (35)$$

where

$$\mu_1 = (\delta + \mu + \rho)\,k_1 = \alpha\,b\,\beta\,\rho + \beta\rho\,\mathrm{s}\,u - c\,r\,u\;\mu_1 k_2 = \frac{g}{r\,g + \beta h} \quad (36)$$

$$k_3 = \sqrt{4\alpha\,b\,\beta\,c\,r\,\rho\;\mu_1 u + {k_1}^2}$$

The first point, E_0, is a virus-free equilibrium point, while the other four points are virus-infected. These four infected equilibrium points are an infected state with no immune responses, an infected state with dominant antibody responses without CTLs, an infected state with dominant CTL responses without antibodies, and an infected state with coexistence responses of both CTLs and antibodies, respectively.

6.4 Stability analysis

Global stability analysis of a dynamical system is a very complex problem. One of the most efficient methods to solve this problem is Lyapunov's theory. To build the Lyapunov function, the technique used in [22, 41], which had been suggested and utilized for other dynamical models, is adopted. The global asymptotic stability of the model for both the uninfected and the infected equilibrium points is investigated and the following stability theorem has been proven:

E_0 *is globally asymptotically stable if* $R_0 \le 1$.
E_1 *is globally asymptotically stable if* $1 \le R_0 \le min\,(\,A_1, A_2)$.
E_2 is globally asymptotically stable if $A_1 \le R_0 \le A_3$.
E_3 is globally asymptotically stable if $A_2 \le R_0 \le A_4$.
E_4 is globally asymptotically stable if $R_0 \ge max\,(A_3, A_4)$.

where.

$$A_1 = 1 + \frac{\beta\,h}{r\,g}, A_2 = 1 + \frac{\beta\,\rho\,b\,(\alpha + \delta)}{c\,r\,u\,\mu_1}, A_3 = \frac{g\rho\,b\,(\alpha + \delta\,)}{c\,r\,u\,h\,k_2\,\mu_1}, \text{and } A_4 = \frac{\beta\,s\,u\,h\,(\alpha + \delta)}{r\,\delta\,(\alpha\,b\,g + \beta\,s\,u\,h\,k_2\,)}. \quad (37)$$

Details can be found in [42].

6.5 Simulations

The model is numerically simulated. *Mathematica 12 program* is utilized to solve the system of ODEs numerically. The simulations are performed using parameters estimated from clinical datasets in [34]: $\beta = 5 \times 10^{-8}$ ml day^{-1}virion^{-1}, $r = 0.01$ day^{-1}, $\delta = 0.14$ day^{-1}, $\alpha = 40$ day^{-1}, $k = 1$ day^{-1}, $\mu = 1$ day^{-1}, $\rho = 8.18$ day^{-1}, $\varepsilon_\alpha = 0.99$,

$\varepsilon_s = 0.56$. The immune system parameters are proposed in [26] as: $u = 4.4 \times 10^{-7}\text{day}^{-1}$, $b = 10^{-2}\text{day}^{-1}, g = 10^{-5}\text{day}^{-1}, h = 10^{-2}\ \text{day}^{-1}, f = 6.4 \times 10^{-4}\ \text{day}^{-1}, q = 2\ \text{day}^{-1}$. The units for s is cells/ml. day^{-1}, and the units for c is day^{-1}. Some parameters are not given in **Table 5**, and the used values will be given explicitly.

Simulations demonstrate the mutual relations between the basic reproduction number, the equilibrium points, and the stability analysis. The variations of all variables $T(t), I(t), P(t), V(t), Z(t), W(t)$ with time are obtained. Each figure represents a case with a specific value for the basic reproduction number R_0 which leads to a corresponding equilibrium point that is stable according to the stability theorem given in the previous subsection. The model under no treatment is simulated first, and it is obtained by assigning $\varepsilon_\alpha = 0$, $\varepsilon_s = 0$, and $k = 1$ in Eq. (24)–Eq. (29). The cases are given in **Table 5**. **Figures 6–10** illustrate that the variables converge to the values of

	s	c	R_0	A_1	A_2	A_3	A_4
case 1	1.3×10^4	22.3	0.733				
case 2	68,225	82.3	1.043	1.05	1.049	1.02	1.07
case 3	1.3×10^5	22.3	7.33	1.005	1.18	36.08	0.204
case 4	1.3×10^5	82.3	1.99	1.05	1.049	1.021	2.036
case 5	1.3×10^6	22.3	73.35	1.005	1.18	36.08	2.036

Table 5.
Cases considered in the simulation.

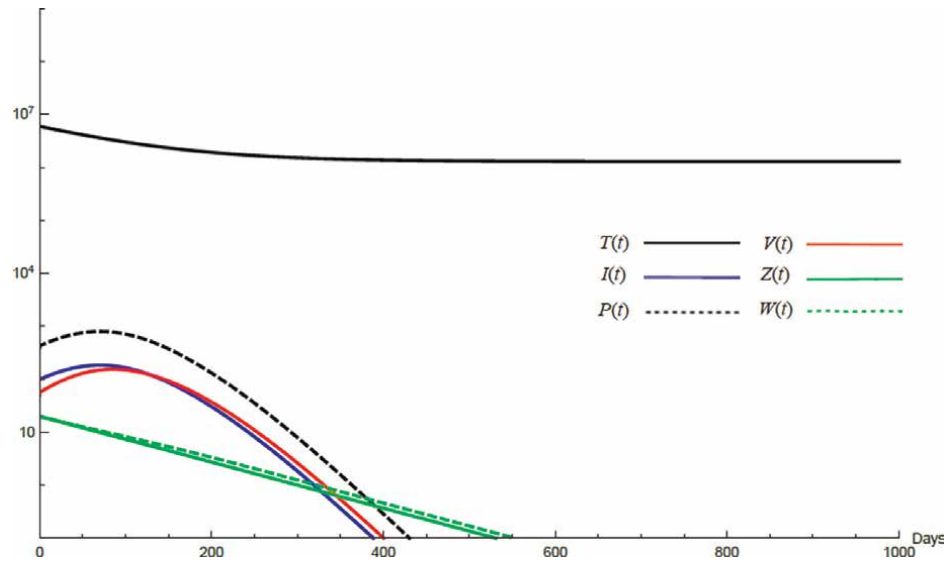

Figure 6.
Variation of the variables for no treatment case 1. Hence, $R_o < 1$ and E_o is stable; $E_o = \{1.3 \times 10^6, 0, 0, 0, 0, 0\}$. $T^o = 0.6 \times 10^7, I^o = 100, P^o = 400, V^o = 50, Z^o = 20, W^o = 20$.

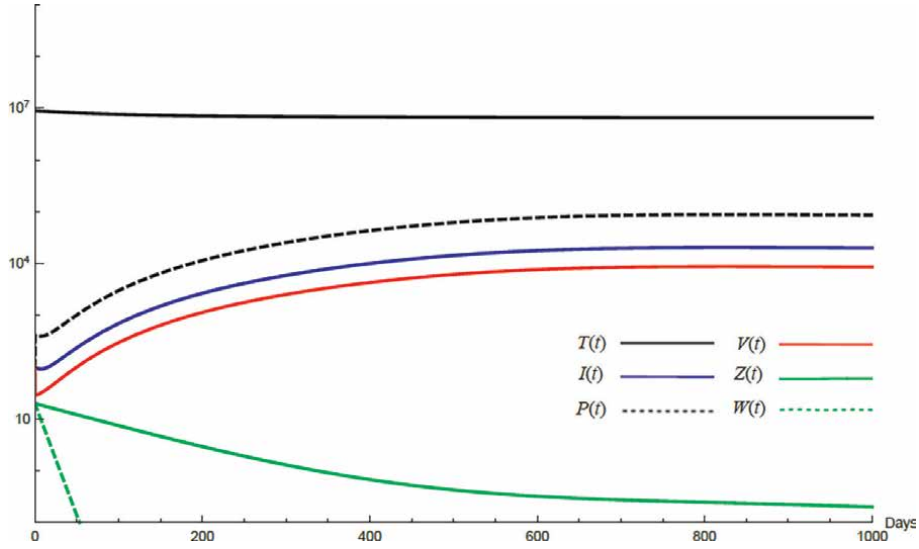

Figure 7.
Variation of the variables for no treatment case 2. Hence, $1 < R_0 < min\,(A_1, A_2)$ *and* E_1 *is stable;* $E_1 = \{6.54 \times 10^6, 20108, 86603, 8608, 0, 0\}$. $T^\circ = 0.9 \times 10^7, I^\circ = 100, P^\circ = 100, V^\circ = 100, Z^\circ = 20, W^\circ = 20$.

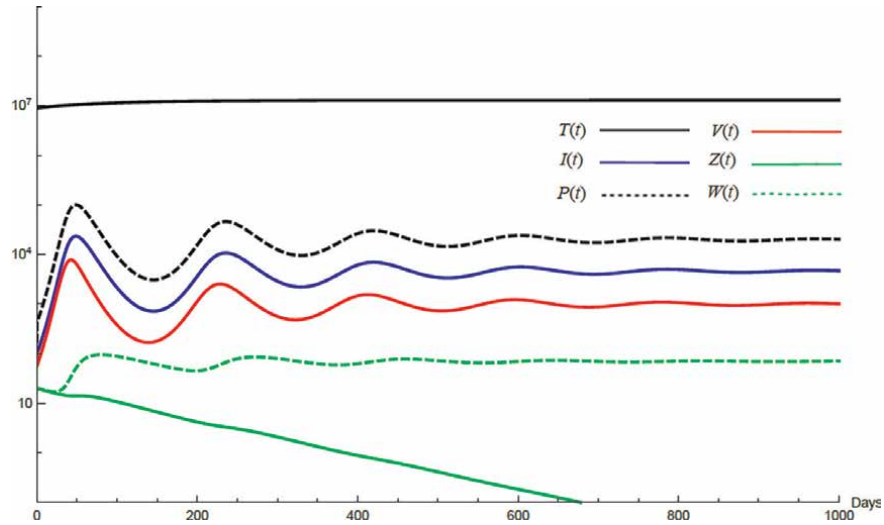

Figure 8.
Variation of the variables for no treatment case 3. Hence, $A_1 < R_0 < A_3$ *and* E_2 *is stable;* $E_2 = \{1.29 \times 10^7, 4620, 19897, 1000, 0, 70\}$. $T^\circ = 0.9 \times 10^7, I^\circ = 100, P^\circ = 100, V^\circ = 100, Z^\circ = 20, W^\circ = 20$.

the corresponding equilibrium point. T^0, I^0, P^0, V^0, Z^0, and W^0 are the initial values of the variables.

To demonstrate the effect of medical treatments, the model with treatment presented by Eq. (24)–Eq. (29) is considered. The same five cases are reconsidered, however, with medical treatment. The effect of medication is reveled in

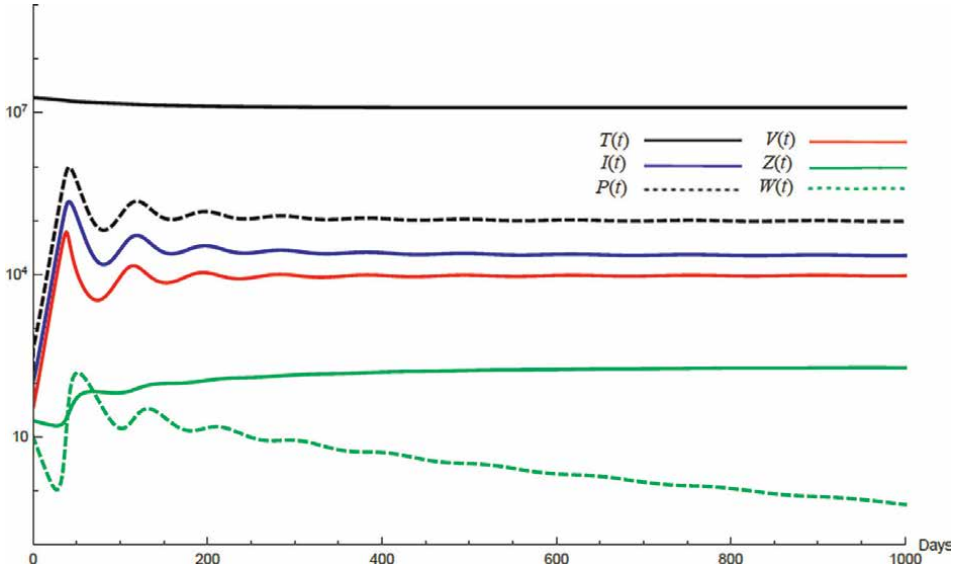

Figure 9.
Variation of the variables for no treatment case 4. Hence, $A_2 < R_0 < A_4$ and E_3 is stable; $E_3 = \{1.24 \times 10^7, 22727, 98191, 9759, 197, 0\}$.$T^\circ = 1.9 \times 10^7, I^\circ = 100, P^\circ = 100, V^\circ = 100, Z^\circ = 20, W^\circ = 20$.

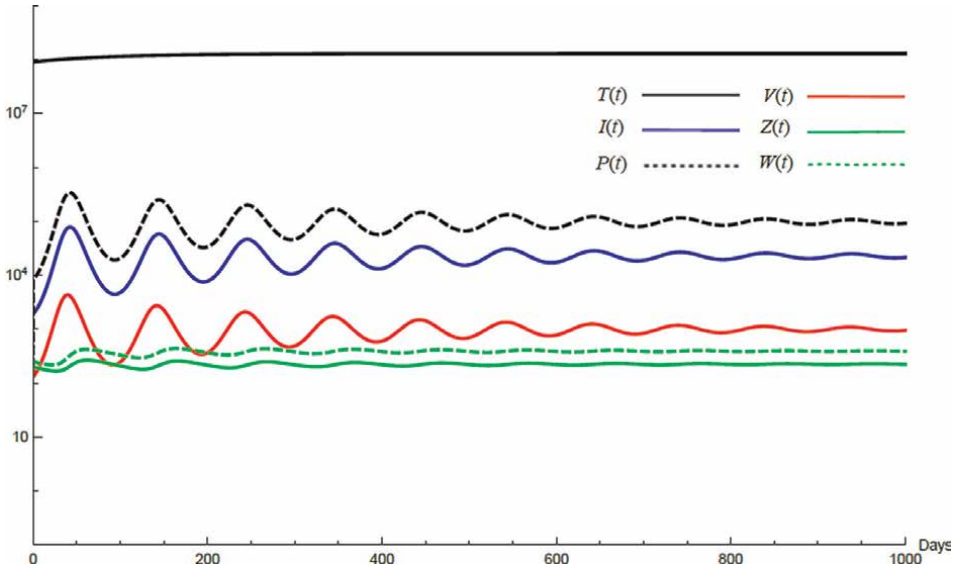

Figure 10.
Variation of the variables for no treatment case 5. Hence, $R_0 \geq max\,(A_3, A_4)$ and E_4 is stable; $E_4 = \{1.29 \times 10^8, 22727, 98236, 1000, 226, 391\}$. $T^\circ = 0.9 \times 10^8, I^\circ = 2000, P^\circ = 200, V^\circ = 200, Z^\circ = 200, W^\circ = 250$.

Figures 11–15. Whenever the curve for the antibodies is not seen, it coincides with the curve for CTLs. The simulation proves the practicality and the effectiveness of the proposed model.

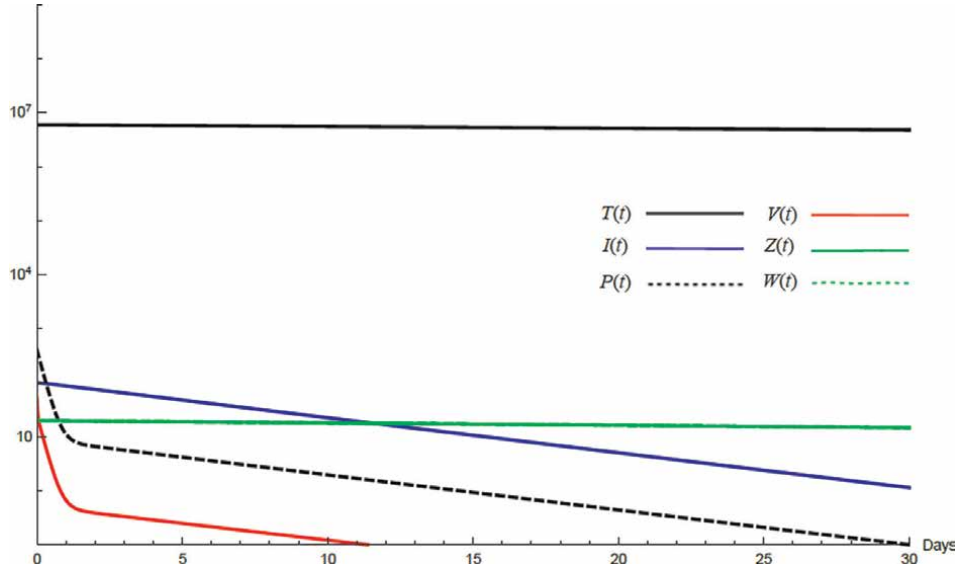

Figure 11.
Variation of the variables for medical treatment case 1.

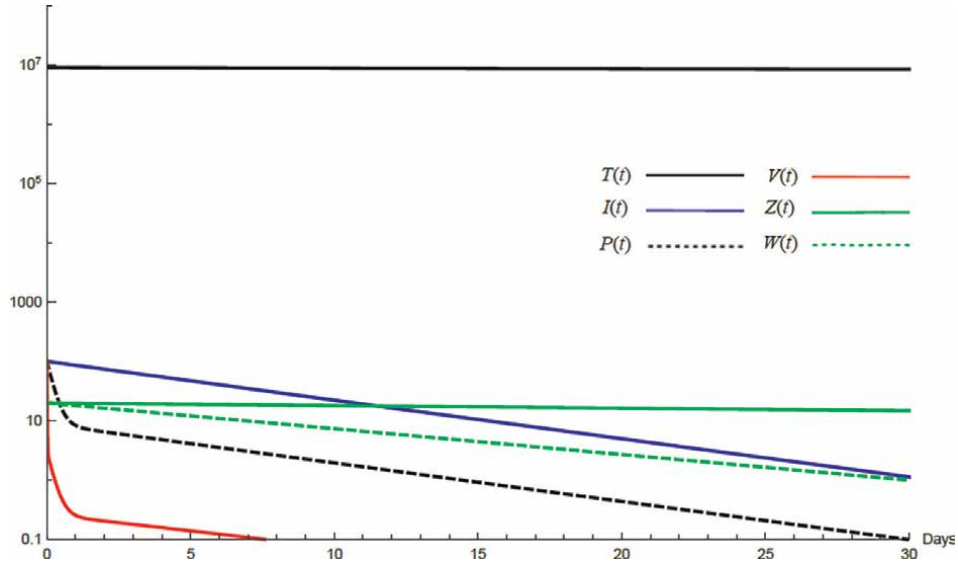

Figure 12.
Variation of the variables for medical treatment case 2.

6.6 Discussion

The parameters related to the immune system do not impact the basic reproduction number, as shown in Eq. (30). When a virus particle is introduced to a population of uninfected cells T, the virus infects some of the cells I and produces intracellular

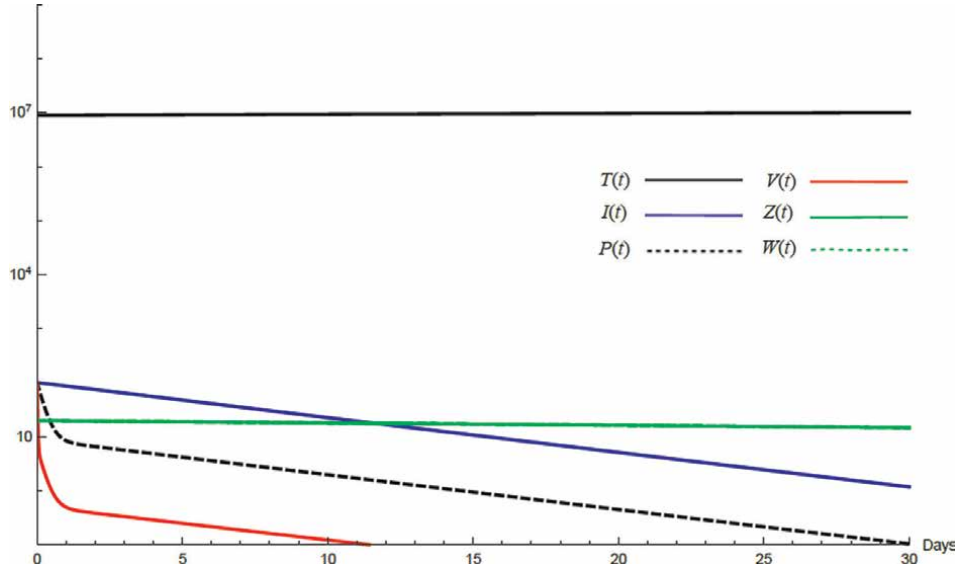

Figure 13.
Variation of the variables for medical treatment case 3.

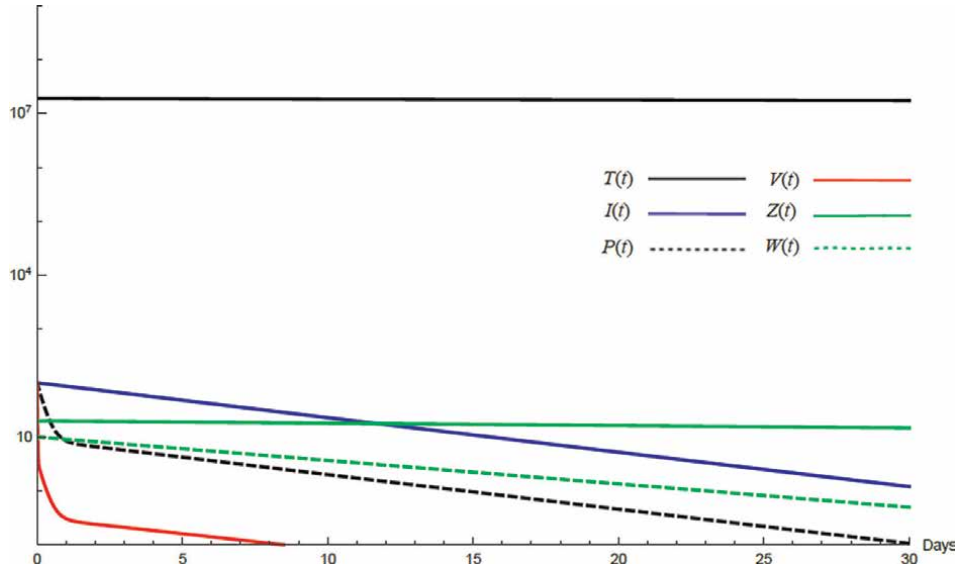

Figure 14.
Variation of the variables for medical treatment case 4.

viral RNA P. However, without the presence of CTLs and antibodies, they cannot be generated. Therefore, the parameters represented by Z and W are not present in the formula for R_0.

There are now five equilibrium points. The first is a virus-free state, and the second is an infected state with no immune response. These two points are the same as those obtained using a model without the immune system [35]. The three infected equilibrium points include a state with dominant antibody response and no CTL

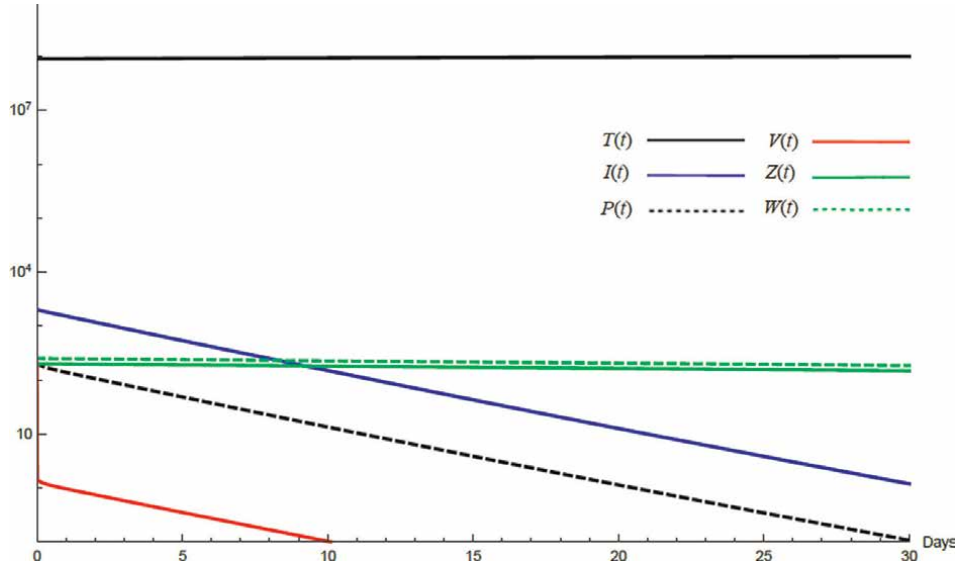

Figure 15.
Variation of the variables for medical treatment case 5.

response, a state with dominant CTL response and no antibody response, and a state with coexistence of both CTLs and antibodies. The CTLs and antibodies are in competition, with the role of CTLs and antibodies in resolving HCV infection being debated in the literature [43, 44]. The infected equilibrium point with dominant antibody response means that the antibody response is strong and reduces the virus load to a level where the CTL response is not stimulated. Similarly, the infected equilibrium point with dominant CTL response means that the CTL response is strong and reduces the virus load to a level where the antibody response is not stimulated. In these two equilibrium points, one of the responses is excluded due to competition between them. The competition may also result in the coexistence of both responses, as seen in the fifth equilibrium point.

Comparing the values of the variables T_*, I_*, P_*, and V_* for the equilibrium point with no immune response E_1 and the equilibrium point with dominant antibody response E_2, it can be seen that that $T_2 \geq T_1$, $I_2 \leq I_1$, $P_2 \leq P_1$, and $V_2 \leq V_1$ when E_2 exists, i.e., $R_0 \geq A_1$. The same is true for the equilibrium point with dominant CTL response E_3 and the equilibrium point with coexistence of both CTLs and antibodies E_4. While the antibody and CTL responses may not completely eliminate the viral load, they significantly increase the number of uninfected cells, decrease the number of infected cells and intracellular viral RNA, and reduce the viral load.

It is important to note that the stability theorem in subsection 6.4 indicates that each equilibrium point has a specific domain of stability. These domains can overlap. For example, if $A_1 \leq A_2$ and $A_3 \leq A_4$, the domains of global stability of E_2 and E_3 will be intersecting except if $A_3 \leq A_2$. This can result in a bistable equilibrium, where two stable equilibrium points coexist. This has been reported in many biological situations, such as in multistrain disease dynamics [40], in low capacity for the treatment of infective in epidemic models [45], and in the investigation of bifurcations and stability of an HIV model that incorporates immune responses [46]. In the presence of bistable equilibrium, the solution converges to one of the two stable equilibrium points depending on the initial conditions, which is called bistable dominance.

The proposed model is a multiscale model that incorporates the immune system response and considers intracellular viral RNA replication and degradation with age dependency in addition to time dependency. This allows the model to explore the dynamics of HCV infection under therapy with DAAs by including both intracellular viral RNA replication/degradation and extracellular viral infection with age dependency in addition to time dependency. The parameter P, which represents the intracellular viral RNA, appears in both the basic reproduction number and the coordinates of the equilibrium points.

7. Conclusion

Power series solution combined with Laplace-Padé resummation method (PSLP) have been used to obtain a general approximate analytical solution for the standard model of HCV for patients treated with DAAs. Results have been compared with experimental viral load data and with published simulated results. The comparison proves that this innovative PSLP solution can be used with confidence for solving the nonlinear standard viral dynamic model. This solution can conveniently be used to fit patient data and estimate parameter values.

An ODEs multiscale model incorporating cell proliferation has been presented. The model has the advantages of both multiscale model and models account for proliferation. Hence, the model includes the major stages in the HCV life cycle that are targeted by DAAs and can also explain some observed behavior in viral kinetic. It can improve our understanding of this biological process including therapy effects. The model eliminates two main limitations in the classical multiscale model and its transformed model, namely, the impossibility to fit the viral load of those patients who show a triphasic profile in virus decay, and lake of representing viral rebound to baseline values after the cessation of therapy. Remarkably, the results of the model have been compared with experimental data reported in the literature, and these results have been fitted to these data.

A multiscale model incorporating the immune system, which plays a crucial role in lowering the virus load, has been developed for understanding and managing chronic HCV. This model offers several benefits over traditional models, including the ability to determine equilibrium points after medication cessation in the presence of immune effects. The parameters related to the immune system do not impact the basic reproduction number, and the disease-free and endemic equilibrium points have been identified. The conditions for their existence have also been determined. The model has a maximum of five total equilibrium points, including the uninfected point, with the four infected equilibrium points being dependent on immune system parameters.

It has been shown that the uninfected equilibrium point is stable if $R_0 \leq 1$ and unstable if $R_0 > 1$. The stability of the infected equilibrium points is based on both the basic reproduction number and the parameters associated with the CTL response and antibody response, which play a critical role in determining equilibrium point stability. Activation of antibodies and CTLs can effectively reduce the viral load but not completely eliminate it.

For effective treatment, if $R_0 > 1$, the focus should be on improving the body's parameters to bring $R_0 \leq 1$. Once this is achieved, treatment to reduce the virus can be implemented until the body reaches the stable uninfected equilibrium point. This will allow the immune system to lead the body to a stable uninfected state. On the other hand, if an unstable uninfected equilibrium point exists, the virus cannot be

eliminated even if the uninfected point is approached. Additionally, successful treatment also means that infected equilibrium points do not exist, so the system will not be drawn to any of them if they were to exist.

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Section 2

Clinical Cases of Hepatitis C

Chapter 5

Current Models to Address Obstacles to HCV Elimination

Brian Conway, Shawn Sharma, Rossitta Yung, Shana Yi and Giorgia Toniato

Abstract

To help inspire global action, the World Health Organization (WHO) has set an ambitious goal of eliminating viral hepatitis, including hepatitis C virus (HCV) infection, as a public health concern by 2030. Globally, an estimated 58 million people have chronic HCV infection, including over 4.5 million people who have recently injected drugs (PWID). Of the 1.5 million new infections occurring per year, over 43% are in this risk group. Systematic approaches are needed with this population to achieve the WHO elimination goals. A number of programs have been successful, most notably in Australia, Scotland, Iceland and North America. We still require additional programs that are easily accessible, multidisciplinary, durable and driven by patient-defined parameters of engagement. We have evaluated housing-based programs as community pop-up clinics to identify HCV-infected vulnerable inner-city residents and offer HCV treatment within such a context. This has been successful, with almost 300 individuals receiving treatment since January 2021, with an effective cure rate exceeding 98%, 99% retention in care, HCV reinfection rates below 1/100 person-years and reduced rates of opioid-related overdose deaths. The implementation of programs, such as ours, must be considered to achieve elimination of HCV infection among PWID on a worldwide basis.

Keywords: HCV infection, illicit drug use, PWID, antiretroviral therapy, interventions, patient engagement, Canada

1. Introduction

Hepatitis C virus (HCV) infections represent one of the leading causes of liver disease and associated morbidity and mortality, affecting over 58 million people worldwide, including over 4.5 million people who inject drugs (PWIDs) [1]. Indeed, drug use (especially injection drug use) currently represents the main risk factor for HCV transmission in the western world. Between 2005 and 2015, the new cases of HCV have increased threefold in this population and roughly 50% of PWIDs have been infected with HCV [2]. In some jurisdictions, the prevalence of HCV infection in drug users approaches 80%, often representing the largest group of infected individuals.

In 2019 it was estimated that there were 171,900 PWIDs living in Canada with an HCV seroprevalence of about 62% [3]. PWIDs make up between 60% and 85% of all incident infections acquired from sharing needles and other drug using equipment [4]. Other risk factors that increase the likelihood of unsafe practices and increased exposure to HCV (but also to other blood borne diseases, such as HIV and HBV) include experiences of homelessness, arrests, incarceration, sex work and mental and emotional instability [5–9]. All these aspects are key factors that need to be addressed when considering optimal HCV treatment strategies for this particular population, in order to maximize engagement in care, including the provision of HCV therapy.

In 2016, the World Health Organization (WHO) set the goal and outlined the strategies for elimination of HCV infection (and also hepatitis B infection) as a public health concern by 2030. For HCV infection, this would require a reduction in the incidence of new infections and mortality by 90 and 65%, respectively, compared to the values reported at baseline (2015). To achieve these targets, many countries have attempted to profile specific interventions to promote HCV elimination. However, many challenges and barriers to HCV eradication remain [10, 11]. Firstly, diagnosis of HCV infection and provision of treatment is difficult because most individuals are asymptomatic, unaware of their disease status and poorly motivated to seek care. Indeed, many wait until disease progression has occurred (advanced cirrhosis, hepatocellular carcinoma and other severe HCV-related complications), limiting the expected benefit of antiviral treatment and increasing disease transmission over years as they remain untreated. Therefore, screening of specific subpopulations with high incidence rates, such as PWIDS, is recommended [11]. Modeling exercises suggest that, simply by addressing HCV in this population, we will reduce new HCV infections by over 40%. The results of such interventions have been mixed, at best. Moreover, the spread of SARS-CoV-2 since the beginning of 2020 and the restrictive measures applied to contain the infection had a direct impact on the health care system in general, especially non-essential services. Among the many consequences of the pandemic and the public health response to it, there has been a disruption of continuity of prevention, treatment and peer-support programs for common conditions among PWID, such as HCV infection, and other consequences, including a significant decrease in harm reduction interventions [12, 13]. Due to all these factors, most countries have fallen behind in their stated objectives to eliminate HCV infection by 2030. In many countries, there has actually been an increase in HCV transmission, especially in vulnerable populations, such as drug users.

It is clear that the HCV pandemic transcends drug users and extends well beyond this group. However, it remains a priority population that must be addressed in a focused manner. Thus, it is crucial to fully understand the barriers to HCV diagnosis and treatment in drug users, design and evaluate interventions and implement evidence informed programs as quickly, effectively and broadly as possible.

2. Successful models of care

Drug users are often not engaged in medical care in the traditional sense or in the usual way. Doing so will require innovation, imagination, and flexibility. Programs will also need to be rigorous and evaluable, such that they can be continuously made more pertinent and effective. Fortunately, there have been a number of successful interventions in many countries and regions from which we can all learn.

2.1 Australia

Since July 2017, in Australia, physicians Matt Young and Joss O'Loan, together with nurse Mim O'Flynn and phlebotomist Mick Mooney, are carrying on a mobile outreach project called the "Kombi Clinic" [14]. The medical personnel don Hawaiian shirts, drives around the greater Brisbane area in an older VW Kombi van fitted with a mobile clinic, bringing life-saving information, and offering free HCV testing and access to treatment where they think they might find people in need of care. The aim of this unique and, many say entertaining, project is to destigmatize HCV infection, by breaking down social barriers, and simplify the access to HCV treatment, thus bringing the offer of cure to the patients rather than expecting them to seek care in the usual way. During the first visit, a doctor provides pre-test counseling and collects medical and social information. Then, a blood test for the diagnosis and evaluation of HCV, HBV and HIV is performed, together with a FibroScan test to assess liver scarring. All participants receive a 10–20 AUD supermarket voucher. On the following visit, performed by telehealth or in person at the next Kombi Clinic, patients found to be productively infected with HCV can actually initiate antiviral therapy. The service is generally accessed by 20–30 clients per week, mainly people who use drugs, people receiving opiate substitution therapy, people experiencing homelessness, indigenous people, people who are, or have been, incarcerated and people living with HIV. The initiative is focused on HCV, but broader health services can be provided, if required. During the period from 2017 to 2020, 25 locations were visited: hostels (22%), homeless drop-in centres (20%), drug rehabilitation services (16%), public festivals (13%) and community centres (12%). A total of 1280 high-risk patients were screened, of whom 453 tested positive for HCV antibodies and 282 were found to be viremic. Of these, 236 were further engaged in care and started HCV treatment. Although 79 were lost to follow up, 103/105 (98%) in whom an outcome was determined are cured. It is quite clear that for these 103 individuals, HCV treatment and cure would never have been achieved without the Kombi Clinic. The challenge for this program going forward is to reduce the rate of loss to follow up so that even more people can benefit from this unique and highly productive initiative.

2.2 Scotland

Professor John Dillon of the University of Dundee has established a program to eliminate HCV (including among all drug users) from the Tayside region of Scotland by 2025. By 2020, his multidisciplinary team had diagnosed 90% of infected individuals patients and treated 80% of this total, already meeting the WHO target for HCV elimination a decade early. In 2017, the National Health Service had launched a trial of "Treatment as Prevention" (TasP), by scaling up HCV outreach and treatment among drug users [15]. The rationale was to use broad-based treatment to lower HCV prevalence among PWIDs to reduce the rate of new infections and re-infections in this population that was, in large part, fueling the pandemic. The key-points of this model were: "keep it local", so patients had to travel as little as possible to achieve a cure of HCV infection; "keep it simple", eliminating any unnecessary tests, assessments, and clinic/hospital appointments; and "keep it known", namely try to deliver the care through someone they already know and trust (including their pharmacist), as that makes it even easier to reach those needing therapy. The programme was carried out in a variety of community-based locations, including conventional clinical settings, drug treatment centres, needle exchange locations, community pharmacies, nurse-led

outreach clinics and prisons. In each location, testing, pre-treatment assessment, and treatment were all provided. The only evaluation requiring travel was liver ultrasound assessment, only needed in those with advanced disease. During treatment scaleup (January 2017 to April 2020), 713 courses of HCV treatment were initiated and were completed in 630 (88.4%) cases. Cure was formally demonstrated in 577 (91.6%) cases. Longer term follow-up after cure was maintained such that 39 cases of reinfection were documented. Re-treatment was completed in 21 cases, with 17 individuals achieving a cure once again. This program is more comprehensive than that of the Kombi Clinic, allowing for the initiation of a larger number of courses of treatment in a variety of community-based settings. Maintenance of engagement in care after cure allowed for the diagnosis of re-infection in a more effective way, and successful retreatment in a number of cases. Here again, an appreciable number of participants (over 10%) did not complete HCV treatment, a group that could likely be re-engaged in care in this structured program going forward.

2.3 Iceland

In 2016, Iceland initiated TraP HepC, a nationwide HCV elimination program. By adopting innovative strategies, the goals for HCV elimination (diagnosing and treating 90% and 80% of infections, respectively) were achieved within a remarkable 3 years [16, 17]. Iceland took advantage of its central registries for infectious diseases, its low threshold for treating addiction, and its national healthcare system to maximize diagnosis, identify potential patients and make treatment access easy for everyone. The program was based on a multidisciplinary approach: nationwide awareness campaigns were promoted, access to testing was improved and harm reduction services were scaled up simultaneously. The priority was given to drug users, but every adult who had chronic HCV infection could participate.

Patients were identified using cross-referencing of four different data sources and, to reach out to those who may be at risk of infection but remained undiagnosed, information was shared through web pages, social media and leaflets, which were sent to every home in the country. The initial evaluation and ongoing care were mainly performed in one of the two involved hospitals, but testing and treatment were provided by staff members through outreach in prisons, homeless shelters and other locations as deemed necessary. To optimize adherence of drug users to treatment, many strategies were implemented, including on-treatment monitoring, pill boxes, increased nurse counseling and support, linkage to other relevant health services (e.g. addiction treatment, psychiatric services), travel stipends for those living outside the city and economic incentives, such as prepaid mobile phone cards. The first visit was comprehensive: review of full medical and social history; completion of all relevant laboratory work; and evaluation of liver fibrosis. Qualified patients started antiviral therapy within 2–4 weeks. Between February 2016 and February 2019, 865 cases were confirmed and 824 were linked to care. Treatment was initiated in 795 cases, with 717 (90.1%) documented cures. This all-of-country approach is particularly impressive, with a unique ability to address the issue of loss to follow up in an effective way.

2.4 United States

In Baltimore, a different, innovative approach was conceived, relying on a peer-based recruitment strategy [18]. An initial group of HCV antibody-positive drug users were interviewed as "primary indexes" to obtain demographic and drug use

information and data about their drug use network. Primary indices were educated on HCV infection, disease and treatment, and then encouraged to recruit other drug users from their network and promote linkage to care. Individuals who presented for engagement (with coupons given to them by their recruiters redeemed for a small financial incentive) were screened by medical staff and, if matching the description and fulfilling all the eligibility criteria, they became secondary indexes and could proceed with treatment and recruitment of their own network. Overall, 17 of 36 primary indexes were able to recruit at least one network member. Of the 64 members recruited, 62 became secondary indexes. Of these, 19 were able to recruit at least one network member. Among all the recruited individuals, 69 participants had chronic HCV infection and were not previously linked to care. Of these, 31 scheduled an appointment, 14 started HCV treatment and 8 completed the therapy. The main barriers for participants that did not schedule an appointment were general health care access and the insurance requirement of a referral from a primary care provider to see an HCV specialist. This program demonstrates the power of peers. If this was included within broader initiatives to address the significant barriers to engagement that were identified, its true benefit could be more fully realized.

2.5 Europe

Four countries (Ireland, United Kingdom, Romania and Spain) are involved in the HepCare project [19, 20]. The aims of this program are to develop, implement and evaluate several interventions to improve HCV diagnosis and treatment among vulnerable populations. The testing and treatment interventions are site-specific, based on the needs of the target populations. Peer support and community- and prison-based treatment are also provided. The HepCare project includes many targeted interventions:

- HepCheck, based on a point-of-care testing strategy through services in the community (opiate agonist therapy clinics, homeless services, prisons). For those testing positive and previously untreated, formal work-up and referrals are offered. Any barriers to treatment are identified and addressed by health professionals and community service staff.

- HepLink aimed to improve linkage to care among those receiving opiate agonist therapy. A trained HCV nurse conducts a full on site work up and facilitates treatment referrals.

- HepFriend, designed to develop and implement peer support interventions within the HepCheck and HepLink work packages. Peers, namely people who with lived experience of HCV infection, drug abuse, and/or homelessness, are recruited and trained to give support within their communities and the specialized services, contributing to integration in HCV care. Peers are also trained to assist the clinical staff in the HepCheck and HepLink activities.

- HepEd, aimed at teaching and delivering educational interventions to vulnerable communities and preparing healthcare providers to collaborate in a shared primary/secondary partnership for HCV treatment.

Overall, 2608 participants were recruited across 218 sites in four European cities (Dublin, London, Bucharest and Sevilla) and HCV antibody test results were

obtained for 2568, 1074 (41.8%) of which were positive. Of 687 viremic individuals, 319 (43.5%) began treatment. To date, among those in whom an outcome has been ascertained, 196/211 (92.3%) are cured. Participants interviewed at all sites said that HepCare improved their HCV treatment experience by decreasing waiting times, giving assistance during the care journey, and providing services in non-traditional locations more suitable to their complex lifestyles. This heroic multicentre approach highlights the benefit of flexible, collaborative and individualized partnerships for HCV elimination. The active inclusion of trained peers further strengthens trust and collaboration between vulnerable people and healthcare providers. In addition, educational interventions help to make affected communities more aware of HCV infection and its possible consequences and the availability of curative treatment.

The strategies discussed above (summarized in **Table 1**) do not represent all those attempted and implemented around the world to address HCV infection among drug users. In some cases, they have led to the elimination of HCV infection from entire countries or regions. In others, they illustrate novel initiatives that, if applied more broadly and incorporated more formally within health care delivery programs, will be important components of HCV elimination. There is clearly a need to be actively present within the vulnerable communities, with low-barrier access to testing (including point of care testing) suitable to the drug using population. It is also essential to provide those with viremic infection prompt engagement in care and access to antiviral therapy. Building a strong collaboration with the drug using community

Model (location)	Strategy	Results	Advantages
Kombi Clinic (Australia)	• Circulating van fitted with a mobile clinic • Free testing and rapid access to HCV treatment • Other health care needs addressed	• 1280 patients screened • 453 HCV Ab+ • 282 HCV RNA+ • 236 treatment starts • 103/105 cured to date	• Receptive environment to remove social barriers • Mobile program bringing treatment to the patients
Treatment as Prevention (TasP) (Tayside, Scotland)	• Keep it local • Keep it simple • Keep it known	• 713 treatment starts • 577/630 cured to date	• Simplification of work-up and access to treatment • broad, multidisciplinary community-based approach
TraP HepC (Iceland)	• Coordinated national program • Priority given to drug users • Large media campaign	• 865 viremic individuals identified • 795 treatment starts • 717 cured to date	• National approach with broad community support • Effective and integrated approach to drug users
Peer-based recruitment strategy (Baltimore, USA)	• Identification of cases through HCV-infected peers	• 69 viremic individuals identified • 14 treatment starts • 8 completed therapy	• Community-based approach • Recognition of the importance of peers in programs aimed at recruiting drug users into HCV care

Model (location)	Strategy	Results	Advantages
HepCare project (Ireland, United Kingdom, Romania, Spain)	Multiple strategies adapted to local needs and priorities • HepCheck (testing) • HepLink (linkage to care) • HepFriend (peer support) • HepEd (education to vulnerable communities and healthcare providers)	• 2568 patients screened • 1074 HCV Ab+ • 687 HCV RNA+ • 319 treatment starts • 196/211 cured to date	• Ambitious approach adapted to multiple and varied local circumstances • Recognition of importance of peers and educational interventions

Table 1.
Comparison of the main HCV elimination strategies attempted in different Countries worldwide.

based on trust and a perceived capability to understand and address needs other than HCV care will also be a key factor in HCV eradication.

3. The Vancouver model

3.1 Current scenario and challenges

We have known for some years that the prevalence of HCV infection within Vancouver's inner city (including the Downtown East Side or DTES) exceeds 60%, particularly among active drug users. Given this large burden of disease and potential for ongoing high-level transmission, understanding the barriers to progression through the care cascade is essential to optimize treatment strategies and enhance HCV care. Compared to the current situation, significant scale-up of treatment rates is required in order to decrease prevalence and have a meaningful impact on onward transmission.

Marginalization of people who use drugs in the DTES of Vancouver has led to their disengagement from health care, including HCV and HIV care. The shift from an individual physician-based approach to a multidisciplinary, community-based intervention may be an important strategy to address this issue. Indeed, many HCV-infected inner-city residents, who are actively using drugs, are facing other issues more challenging than their HCV infection: housing and financial insecurity, untreated mental illness, and active untreated addiction. In this context, an approach that targets their medical, social, psychological, and addiction-related needs, and one that focuses on engagement, multidisciplinary and durability are required to identify candidates for HCV treatment and provide them with antiviral therapy in a way that is accountable, generalizable and scalable. Several strategies have been proposed to identify HCV-infected inner-city residents, engage them in care, provide them with antiviral therapy, establish conditions to maximize treatment completion and the achievement of cure. Despite the improvements in linkage to diagnosis and investigations to treatment, in our city of Vancouver, initiation of treatment and, even more, completion of HCV therapy for this population are still critical, especially in light of a significant opioid crisis with 7 overdose deaths/day, largely among individuals disengaged from care.

3.2 Operationalizing an innovative, multidisciplinary approach – the community pop-up clinic (CPC)

It is in this context that we have developed our highly successful Community Pop-Up Clinic (CPC) model aimed at reducing barriers to treatment and maximizing HCV therapy initiation in the inner-city population. In weekly events held at various inner-city locations (mainly single room occupancy housing projects, each with 30–90 residents on average), we interact with up to 30 residents/event. If HCV infection is present (determined by review of provincial records or ascertained by point-of-care testing offered on site), immediate engagement in care is offered. This will address social, psychiatric, medical, and addiction-related needs and deliver HCV therapy in this context. During the CPC event, an immediate consultation with a nurse or doctor is offered, with follow-up within the week at our centrally located clinic, with prompt initiation of HCV therapy in this context focused on achieving and maintaining engagement, ensuring that it is both multidisciplinary and durable, according to individual need. If someone does not attend the follow-up appointment, we would implement short-term strategies, such as return visits to the place of residence using our medical van (with subsequent provision of HCV treatment at a distance) or alternate strategies for follow up.

In summary, the program developed at our Vancouver Infectious Diseases Centre (VIDC) provides ongoing, long-term access to specialty medical care and support services in order to target the clinical, psychological, and social factors along with addiction-related needs that impact drug users, particularly the population from the DTES.

From January 2021 to November 2022 (23 months), we conducted 80 CPCs and evaluated 1440 individuals. Of these, 477 individuals (33.1%) were found to carry HCV antibodies, with 331 (69.4%) found to be viremic. We attempted to engage all in broad-based care to treat their HCV infection. To date, engagement has been secured in 289 (87.3%) cases (**Figure 1**). At this time, 252 have started treatment and

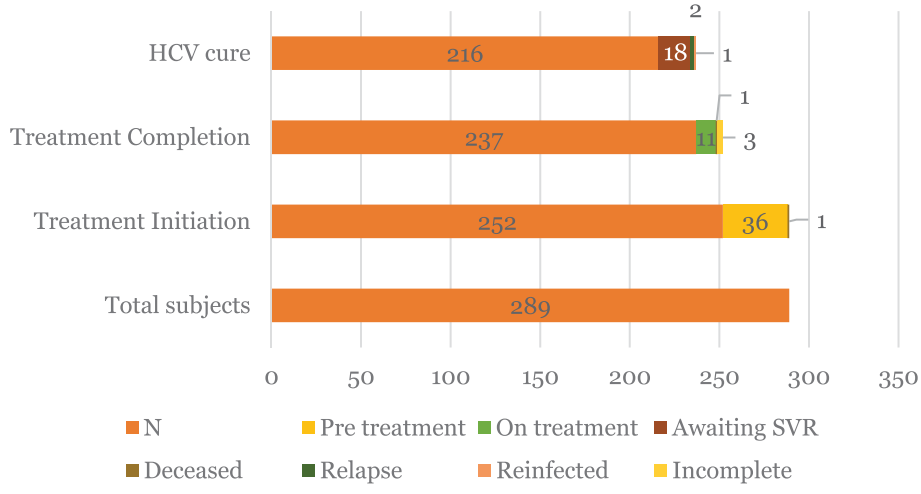

Figure 1.
Cascade of care – Community Pop-Up Clinic (CPC) Program (2021–2022). Through our CPC model, from January 2021 to November 2022, we effectively engaged in treatment 289 subjects. HCV cure was confirmed in 216 cases out of 224 (96.4%) in whom a definite outcome has been established.

one individual died of an opioid overdose in the pre-treatment phase. Of these, 11 remain on treatment, only 3 have been lost to follow-up and one additional individual succumbed to an opioid overdose. Of the 237 who have completed treatment, a definitive outcome has been ascertained in 219 cases, with 216 cures, 2 virologic relapses and one early reinfection. The effective cure rate among the 224 individuals in whom a definite outcome has been established is 96.4% (216/224). It is worth noting that in this vulnerable population where 7 opioid overdose deaths/day in the community, we only documented 2 overdose deaths in our cohort.

The classical cascade of care (**Figure 2**) of HCV diagnosis and treatment has been defined according to four specific parameters:

1. Initial engagement.

2. Treatment preparation.

3. Treatment phase.

4. HCV cure confirmation.

Thinking of our program as one that means to identify viremic individuals (many of whom have been diagnosed in the remote past), a conceptual re-definition of the cascade may allow us to define and monitor a more meaningful measure of its success. Thinking of the steps to cure HCV infection from the initial interaction at a CPC event, the cascade becomes (**Figure 3**):

1. Time from initial engagement to treatment initiation.

2. Initiation and completion of antiviral therapy.

3. Ascertainment of treatment outcome.

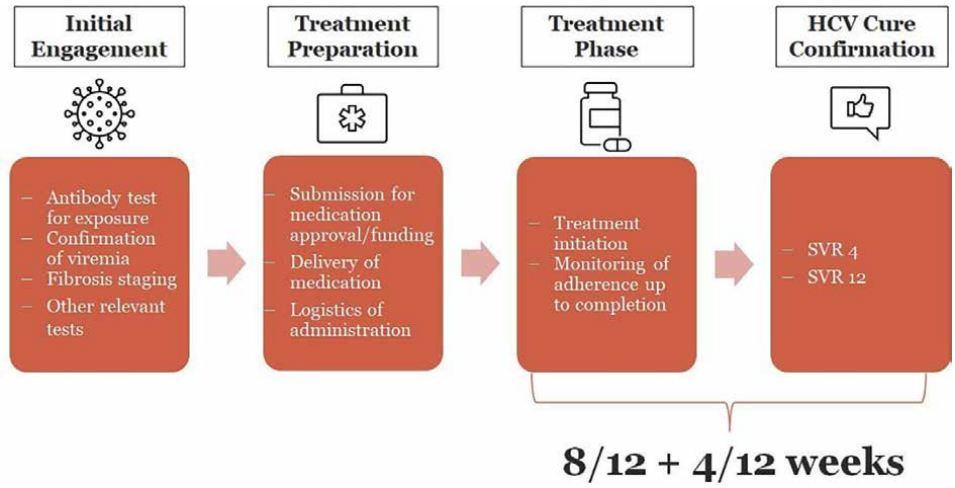

Figure 2.
Classic cascade of care. The classic cascade of care involved 4 consecutive phases, each of them involving further specific steps.

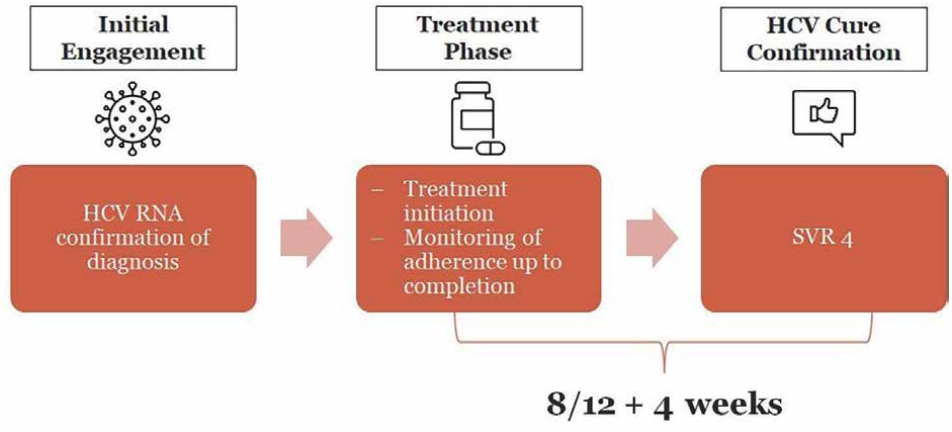

Figure 3.
"Care to cure (C2C)" initiative. The "Care to cure (C2C)" initiative represents a simplified model of cascade of care elaborated by understanding the critical steps of the traditional strategy in order to allow a greater engagement and treatment of PWIDs.

This functional cascade would allow us to evaluate another aspect of program performance, as a more efficient progression towards cure would likely serve to increase the strength of engagement and allow for more individuals to be treated over time. The duration of treatment is a fixed number of weeks, most usually 8–12 weeks. Current data seems to suggest that the absence of viremia as soon as 4 weeks after the end of treatment almost always predicts cure. Programs such as ours are ideally designed to maintain individuals in care once therapy has started and following its completion. Our data shows a loss to follow-up rate of 1–2%, which supports this assertion. The variable on which we can act is the progression from initial contact to treatment initiation. In the cohort presented above, the median time was 6 weeks. This needs to be maintained or improved going forward, and this will be a key priority for us in the coming years.

Taken together, the data we present validates the development of multidisciplinary programs, such as ours aimed at treating HCV in vulnerable populations that must be engaged in care for HCV elimination to become a reality and documents additional societal benefits that could be achieved from such a program. This is a highly successful initiative on several fronts. The majority of individuals would not have received HCV treatment without this initiative, with many having had documented viremia several years before. The high success rate of therapy shows that, if treatment is delivered within a system appropriate for the specific patients, it can be as successful as has been demonstrated in clinical trials, but in a much more challenging population. In addition, our very low rate of loss to follow-up shows our success in the process of engagement strategies.

However, our program does have certain limitations. Some patients remain untreated. After we have determined that they are viremic and eligible for treatment, in some cases we are unable to proceed. The structure and size of our program are such that comprehensive follow-up may not always be possible, especially if the individual's status has changed. This prolongs the time from care (our knowledge that an individual is viremic) to treatment and reduces our ability to eliminate HCV in this key population.

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4. Conclusion

When it was initially put forward in 2016, the goal of viral hepatitis elimination (including HCV infection) over the next decade and a half seemed both aspirational and attainable. As we are now halfway between 2016 and 2030, it appears to be more daunting than we could have imagined. Of course, there has been a pandemic that has affected our ability to set up and maintain effective programs. Despite this, we can report a number of success stories in many parts of the world, including innovative approaches to drug-using populations. However, such programs have been largely driven by local champions and adapted to local needs. To our minds, there remains the need to develop, implement and evaluate strategies that are community-based and provide HCV treatment in a broader context, where all the needs of the individual that is before us are met in a coordinated fashion. The initial goal of such programs must be engagement in care and first meet the priority needs of the population, that include management of addiction in the context of an opioid overdose crisis, in our community and in many others in the developed world. The programs must be accountable, both to the community we serve and to public health authorities and other funding agencies. They must be subjective to rigorous evaluation, to celebrate their successes and identify challenges that must be addressed to improve their impact and effectiveness. They must also be scalable, to address the needs of all PWIDs and vulnerable groups throughout the world. Elimination of HCV infection must be viewed as a partnership between those who provide care and those who receive it. Partnership with inner city populations is particularly complex but attainable. If we do the right things in the right way at the right time, we will not only eliminate HCV, but improve the health and well-being of the most vulnerable among us and contribute in a significant way to improve society as a whole. There is no higher calling.

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Conflicts of interest

Dr. Conway has received research grants, honoraria and/or acted as a remunerated advisor for AbbVie, Astra Zeneca, Gilead Sciences, GSK, Indivior Canada, Merck, Moderna, Sanofi Pasteur, Seqirus, and ViiV Healthcare. In particular, AbbVie and Gilead Sciences have funded the Community Pop-up Clinic program in a direct way.

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Chapter 6

Chronic Hepatitis C Virus Infection in Chronic Kidney Disease

Gde Somayana and Komang Agus Wira Nugraha

Abstract

Chronic hepatitis C virus (HCV) infection in chronic kidney disease (CKD) patients can accelerate the decline of kidney function, increase the risk of kidney failure, and increase mortality in CKD patients on hemodialysis (HD). Chronic HCV infection is also a risk factor for mortality in kidney transplant patients. Effective detection, evaluation, and treatment for HCV infection can improve kidney and cardiovascular outcomes. In the subsequent 10 years, direct-acting antivirals (DAAs) have become available. DAAs enabled a greater rate of HCV eradication in CKD populations. Patients with stage 1-3b CKD (G1-G3b) can be treated with any licensed DAA regimens. The recommended DAA treatment regimens for CKD stage 4–5, including those undergoing HD (G4-G5D), are the sofosbuvir-free combination therapies (grazoprevir/elbasvir and glecaprevir/pibrentasvir). While sofosbuvir-based regimens are much more accessible, data showed that some countries have limited access (due to drug availability and high cost) to sofosbuvir-free regimens. Because of this phenomenon, some countries have had difficulty providing sofosbuvir-free treatment to CKD G4-G5D patients. As an alternative to those conditions, some clinicians have approved the usage of sofosbuvir-based regimens in CKD G4-G5D, but this decision is still debatable. Kidney Disease: Improving Global Outcomes (KDIGO) 2018 did not approve sofosbuvir-based regimens for CKD G4-G5D. On the contrary, other studies and guidelines have approved sofosbuvir-based regimens for CKD G4-G5D patients.

Keywords: hepatitis C virus, chronic kidney disease, DAA treatment, hemodialysis, kidney transplant

1. Introduction

Hepatitis C virus (HCV) infection is a global health problem that leads to liver cirrhosis, liver decompensation, and hepatocellular carcinoma (HCC) [1]. HCV infection and chronic kidney disease (CKD) are epidemically correlated [2]. There is a link between HCV and CKD; thus, on the one hand, HCV can cause CKD through mixed cryoglobulinemia in the renal matrix or through the development of glomerulonephritis. On the other hand, CKD (especially in stage 5 CKD) is a risk factor for HCV as many patients receive blood transfusions, hemodialysis (HD), or develop a donor-derived infection after kidney transplantation [1, 3].

Chronic HCV infection in CKD patients can accelerate the decline of kidney function, increase the risk of kidney failure, and increase mortality in CKD patients with

HD [4]. Chronic HCV infection is also a risk factor for mortality in kidney transplant patients. Other post-transplant complications in untreated chronic HCV infection patients are diabetes mellitus, chronic allograft nephropathy, rapid graft loss, and lymphoproliferative disorders [3, 5–7].

Effective detection, evaluation, and treatment for HCV infection can improve kidney and cardiovascular outcomes [4]. In the subsequent 10 years, the development of direct-acting antivirals (DAAs), which enabled a greater rate of viral eradication in CKD populations infected with HCV, has become available [4, 8]. Patients with stage 1-3b CKD (G1-G3b) can be treated with any licensed DAA regimen [8–10]. Several regimens also have been approved for use in patients with HCV infection and stage 4–5 CKD (G4-G5), including those on dialysis (G5D) [4]. The recommended DAA treatment regimens for CKD G4-G5D are the sofosbuvir-free combination therapies that consist of grazoprevir/elbasvir and glecaprevir/pibrentasvir [8, 10].

Unfortunately, the availability and cost of sofosbuvir-free therapies have become a problem for low- and middle-income countries. Most countries can only access sofosbuvir-based therapies. In 2019, a total of 62 low- and middle-income countries had registered at least one version of sofosbuvir/daclastavir, sofosbuvir/velpatasvir, or sofosbuvir/ledipasvir. Low- and middle-income countries can now aim for a 12-week course of treatment with WHO-prequalified generic sofosbuvir and daclatasvir for as little as US$ 60 per patient [11]. Therefore, high cost and limited drug availability of certain DAA regimens are the major barriers to achieving HCV eradication in CKD patients [4].

Sofosbuvir-based regimens are mainly eliminated through the renal route and have been initially licensed for patients with a glomerular filtration rate (GFR) above 30 mL/min [12–14]. Its use in patients with CKD G4-G5D is not indicated in label [12]. On the contrary, several studies found that sofosbuvir-based regimens were effective and safe in patients with GFR ≤30 mL/min [12, 15]. All DAAs are now recommended by the American Association for the Study of Liver Diseases (AASLD) for GFR ≤ 30 mL/min [15]. Based on the controversies and growing studies of DAAs treatment, this review will summarize the epidemiology, detection, evaluation, and recommended DAAs treatment in CKD patients. The role of sofosbuvir-based regimens in CKD G4-G5D patients will also be briefly discussed here. Finally, we hope this review could help us to create optimal strategies aimed at improving the quality of care and overcoming drug availability or cost barriers for CKD patients.

2. Epidemiology of chronic HCV infection in CKD

HCV is a common infection in CKD patients, with a prevalence rate of 10–16% worldwide [16]. The serum anti-HCV and HCV ribonucleic acid (RNA) have been detected in a significant proportion of patients with CKD [1]. According to the Dialysis Outcomes and Practice Patterns Study (DOPPS, 1996–2005), HCV prevalence in HD patients was 9.9% overall [17]. Among patients with HD, its prevalence in Belgium is 4%, about 20% in the Middle East, and an intermediate prevalence in Italy, Spain, China, Japan, and Russia [17–19]. In Taiwan, Malaysia, South Korea, Thailand, Singapore, and Hong Kong, the prevalence of HCV infection in HD patients is 13, 4, 4, 3.96, 2.1, and 0.9% [4]. In the United States (US), the prevalence of chronic HCV infection in HD patients has been estimated to be five times higher than in the general population [3]. HCV prevalence was higher among US black patients compared with US non-black patients (8.9 vs. 4.1%) [17].

The characteristics of patients associated with the prevalence of HCV infection in prevalent patients undergoing dialysis included younger age; US black race; longer dialysis vintage; history of hepatitis B virus (HBV), human immunodeficiency virus (HIV) positivity, and cirrhosis; substance abuse; and glomerulonephritis as the etiology of end-stage renal disease. Patients with HCV infection had an unadjusted lower prevalence of coronary artery disease (CAD), hypertension, peripheral vascular disease, diabetes mellitus, congestive heart failure (CHF), lung disease, cerebrovascular disease, and cancer, which reflects the younger age of HCV infection patients [17].

HCV incidence was 1.2 per 100 patient-years in the DOPPS 5 study (2012–2015) and ranged from 0 in Belgium, Sweden, and Turkey to 2.9 in Italy. Patients who had HD for more than 10 years had higher rates of HCV seroconversion (seroconversion among patients with an initial negative HCV antibody measurement). HBV-positive and HIV-positive patients also had a higher rate of HCV seroconversion. The use of HD isolation stations for chronic HCV infection patients was associated with lower rates of HCV seroconversion, although the association was not significant statistically [17].

The annual incidence of HCV infection in patients with HD has decreased from 2.9 to 1.2% from 1996 to 2015. But on the other side, this annual incidence remains much higher than the global incidence of 23.7 per 100.000 in the general population [17–19]. The higher incidence and prevalence rate of HCV infection in patients with GFR $\leq$ 30 mL/min compared to the general population can be attributed to three factors. First, there is a direct association between HCV infection and cryoglobulinemic nephropathy, membranoproliferative glomerulonephritis, or membranous glomerulonephritis. HCV is also associated with insulin resistance, diabetes mellitus, and cardiomyopathies, which could worsen kidney function. Second, the risk of nosocomial HCV infection increases in patients with CKD receiving kidney replacement therapy [1]. In kidney failure patients, dialysis modality is an independent risk factor for acquiring HCV infection, with HD being associated with a higher risk of HCV infection than peritoneal dialysis (PD). HCV seroprevalence and seroconversion rates among HD patients vary widely, suggesting a need for consistent, rigorous local infection control measures [4]. Third, HCV infection may occur as a result of donor-derived infection following kidney transplantation [1, 3].

3. Natural history of HCV infection in CKD

If acute HCV infection is not treated, approximately about 65.4–92% of patients on maintenance HD develop chronic infection. Making an early diagnosis is difficult because most infected patients are asymptomatic, and have serum alanine transaminase (ALT) levels below the reference limit for subjects without advanced kidney diseases. This condition also makes determining the precise duration of HCV infection difficult for clinicians [20–22].

Current evidence indicate that the course of HCV infection is less aggressive in patients on hemodialysis than in nonuremic patients. Studies about the effects of HCV genotypes on the progressivity of CKD remain controversial, but HCV viremia is associated with progressive kidney failure [23–25]. According to REVEAL-HCV studies, patients with HCV genotype 1 infection tended to develop end-stage renal disease. On the contrary, patients with HCV genotype 2 infection are more likely to develop CKD stage 2 or higher [1].

Chronic HCV infection in CKD patients can increase the risk of mortality [4]. Data showed that mortality is a firm outcome of the natural history of HCV infection [1]. A meta-analysis reported that the pooled adjusted hazard ratio for all-cause

mortality in HCV-positive patients undergoing hemodialysis was 1.207 (95% CI 1.12–1.30, $p < 0.001$; $I^2 = 75.59\%$) compared with HCV-negative patients [26]. Other meta-analysis revealed the summary estimate for adjusted death risk (all-cause mortality) with anti-HCV antibody across the retrieved studies was 1.26 (95% CI: 1.18–1.34) ($P < 0.0001$) The overall estimate for adjusted death risk (liver disease-related mortality) was 5.05 (95% CI: 2.53–10.0) ($P < 0.0001$) [27]. On the other hand, dialysis patients with active HCV infection who undergo kidney transplantation have better survival compared with patients on maintenance dialysis [1]. Unfortunately, the graft survival rates in kidney transplantation recipients with HCV infections are worse than those in kidney transplantation recipients without HCV infections [28].

4. Detection and evaluation of HCV infection in CKD

The first step in the evaluation of chronic hepatitis C in CKD or end-stage renal disease (ESRD) patients is similar to that of other patients in the general population. The evaluation should include biochemical liver function test for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT). Because of pronounced viremia reduction mediated by HD-induced activation CD69+ lymphocytes and an increased serum level of alpha-interferon (IFN-α), chronic hepatitis C in ESRD patients tend to show normal or mildly elevated liver enzymes [28]. Hemodialysis is also responsible for increasing the production of hepatocyte growth factor (HGF), which could stimulate hepatocyte turnover and liver regeneration. HGF plays a protective role against exogenous toxins and is associated with the indolent course of liver disease [28, 29]. The effect of increased HGF may account for less severe histological liver findings. Albumin, bilirubin, platelet count, and prothrombin time (PT) examinations can also reflect liver fibrosis progression. Low platelet and prolonged PT are the signs of advanced liver fibrosis or the development of portal hypertension [28].

Patients with CKD, including those initiating kidney replacement, should undergo screening for HCV. A positive anti-HCV antibody can indicate the presence of an active infection (chronic or acute), the resolution of a past infection, or a false positive. The presence of HCV antibody is not protective against HCV infection, because the virus can escape the host's immune response even in the presence of HCV antibody. The clinical relevance of positive HCV antibody is limited as a marker of exposure to HCV and persists even after the patient achieves sustained virologic response (SVR) or cure [3]. Therefore, the detection of anti-HCV antibody by screening immunoassays with infection confirmed by nucleic acid testing (NAT) is required to make a diagnosis. In HD patients, the positive predictive value (PPV) of anti-HCV antibody was 73% and the negative predictive value (NPV) was 90%. HCV-RNA was positive in 10% of anti-HCV-negative patients. This figure results from the delay between viremia after contamination and seroconversion (the "window period"). As a result, initial NAT testing should be done in HD units with a high HCV prevalence. Because HD can reduce the level of viremia, NAT blood samples should be drawn before dialysis. However, this reduction in HCV RNA is not dependent on the dialysis schedule or type of membrane used [10].

Anti-HCV antibody will remain the initial screening test for patients with HD, because of its low cost. The HCV core antigen test could be an alternative if the NAT test could not be performed. But for some countries, the HCV core antigen is not yet available and is expensive. Given that HCV core antigen has a detection threshold of

at least 3000 IU/mL, and most patients with HCV have high levels of viremia, good concordance between HCV core antigen and NAT has been demonstrated [4].

In immunosuppressed patients (HIV-positive, chemotherapy, or transplant recipients), a HCV RNA test needs to be performed even if the anti-HCV antibody is negative [3]. A study in German reported an overall prevalence of 0.8% of HCV RNA-positive subjects, although HCV antibody levels were negative [30]. An HCV genotype test is still recommended if the patient has detectable HCV viremia to personalize appropriate treatment choices. It is also important to consider potential resistant associated substitutions (RAS) before starting treatment in specific conditions [3].

All HD patients should be screened for HCV infection using the ALT level monthly and the anti-HCV antibody or NAT every 6 months. If a newly acquired HCV infection is detected, all patients should be screened and the testing frequency should be increased [10]. Some countries perform the NAT test annually for patients who either are HD and anti-HCV positive [4].

The cirrhosis condition carries an increased risk of the development of HCC, liver transplantation, or mortality. Guidelines from Kidney Disease: Improving Global Outcomes (KDIGO) 2018, recommended the use of non-invasive markers of liver fibrosis, and liver biopsy can be performed if non-invasive markers are not conclusive or to rule out other liver-related comorbidities. Due to recurrent anticoagulation and uremic platelet dysfunction in HD patients, liver biopsy carries a lot of risks [3].

Transient elastography (fibroscan), AST platelet ratio index (APRI), fibrotest/fibrometer, and Fibrosis-4 (FIB4) index are recommended as an initial test for staging liver fibrosis. Transient elastography measures shear wave velocity from a transducer at the end of an ultrasound probe passing through the liver. The velocity is then converted into liver stiffness measurement and expressed in kilopascal (kPa). A study about the validation of transient elastography in chronic hepatitis C patients with dialysis reported that transient elastography was better than APRI for fibrosis stage $\geq$ F2 and $\geq$ F3. The difference was not statistically significant in stage F4, probably because of the small number of cirrhotic subjects in the population of the study [31]. The severity of portal hypertension needs to be confirmed after cirrhosis is suspected or confirmed. Endoscopy is indicated to confirm esophageal varices [10].

5. Treatment of HCV infection in patients with CKD

Multiple studies have found an association between sustained SVR and a reduction in ESRD-related mortality, a reduction in vascular events, an improvement in cryoglobulinemic vasculitis, and a reduced risk of deterioration in kidney function [10]. A recent KDIGO clinical guideline recommended that all CKD patients infected with HCV should be evaluated for antiviral treatment [4, 8, 10]. Interferon-free regimens should be applied in CKD patients because interferon-based therapy is associated with low efficacy (SVR rates of 37–41%), poor tolerability, and acute rejection of graft or allograft loss in kidney transplant recipients [8, 10, 32]. Ribavirin was also used with caution in CKD patients due to its proclivity to cause anemia [10].

DAA is the backbone of treatment for HCV infection in CKD patients. The choice of specific DAA regimens is based on HCV genotype, viral load, treatment history, drug interactions, GFR, liver fibrosis stage, and kidney or liver transplant candidacy [8]. All CKD patients also should undergo evaluation for HBV infection (hepatitis B surface antigen/HBsAg and anti-hepatitis B core/anti-HBc) before beginning DAA treatment [4, 10]. If hepatitis B surface antigen (HBsAg) is detected, HBV antiviral treatment

should be considered to prevent HBV reactivation as a consequence of DAA treatment. If there is a history of resolved HBV infection, clinicians should monitor HBV reactivation during DAA treatment (using serial HBV DNA and liver function tests) [10].

The classification of CKD is important to determine before starting DAA treatment. CKD is classified on the basis of cause, GFR category (G1 to G5), and albuminuria category (A1 to A3, presented as albumin-creatinine ratios). **Figure A1** shows the classification and its prognosis, as used by KDIGO [10].

5.1 Treatment of HCV infection in patients with CKD GFR category G1 to G5 and G5D

The KDIGO and European Association for the Study of the Liver (EASL) recommended that patients with CKD G1-3b be treated with any licensed DAA regimens [4, 8, 10, 33]. No dose modification is necessary for most DAA regimens. Protease-inhibitors (telaprevir, boceprevir, simeprevir, paritaprevir, sovaprevir, asunaprevir, faldaprevir, glecaprevir) are contraindicated in liver cirrhosis patients because of risk of hepatic decompensation or hepatotoxicity [10]. **Table 1** shows the treatment options for HCV infection in patients with CKD according to the KDIGO HCV in CKD guideline.

KDIGO recommended that CKD patients with GFR below 30 mL/min/1.73 m^2 (CKD G4-G5D) be treated with a ribavirin-free DAA-based regimen. For patients with CKD G4-G5D, the first-line treatment recommendation is either glecaprevir/pibrentasvir for all HCV genotypes or grazoprevir/elbasvir for genotypes 1 and 4. For patients with CKD G5 who are receiving PD, no evidence is available; therefore, following the proposed regimens for patients receiving HD is reasonable [8].

The EXPEDITION-4 study's phase III trial found that using glecaprevir/pibrentasvir in 104 CKD G4-G5 patients resulted in SVR12 rate of 98% (SVR after 12 weeks post-treatment) [34]. In the nonrandomized multicenter EXPEDITION-5 study of CKD G3b-G5 patients with HCV infection, it was shown that glecaprevir/pibrentasvir treatment yielded a high SVR12 rate (97%) [35]. In a prospective study of the Japanese

Kidney function	Regimen(s)	Strength of evidence
CKD G1-G3b	Any licensed DAA regimen	1A
CKD G4-G5, including HD		
• HCV genotypes 1	Grazoprevir/elbasvir	1B
	Glecaprevir/pibrentasvir	1B
• HCV genotypes 2	Glecaprevir/pibrentasvir	1B
• HCV genotypes 3	Glecaprevir/pibrentasvir	1B
• HCV genotypes 4	Grazoprevir/elbasvir	2D
	Glecaprevir/pibrentasvir	1B
• HCV genotypes 5	Glecaprevir/pibrentasvir	2D
• HCV genotypes 6	Glecaprevir/pibrentasvir	2D

**Recommendation grades (1 or 2) and strength of evidence (A to D) are provided for each recommended regimen and HCV genotype. Level 1, "we recommend"; level 2, "we suggest". Grade A, high quality of evidence; B, moderate quality of evidence; C, low quality of evidence; D, very low quality of evidence [8].*

Table 1.
Treatment options for HCV infection in patients with CKD according to the KDIGO HCV in CKD guideline [8].

population assessing 8 or 12 weeks of glecaprevir/pibrentasvir treatment, SVR was achieved in 100% of patients with CKD G4, 99% of patients with CKD G5, and 99% of patients on HD [36]. A study about the safety and efficacy of glecaprevir/pibrentasvir in 2238 CKD patients with HCV infection genotype 1–6, reported an overall SVR rate of 98%, with no difference between CKD G1-G3 or G4-G5 [37].

Grazoprevir/elbasvir also demonstrated promising results in CKD patients. A randomized controlled trial of grazoprevir/elbasvir in CKD G4-G5 with HCV genotype 1, reported an SVR rate of 94% in patients who received immediate treatment and an SVR rate of 98% in patients with postponed treatment [38, 39]. In a quasi-experimental study of CKD G5D in Bandung (Indonesia), grazoprevir/elbasvir was effective in reducing liver fibrosis degree (based on the APRI score) [40].

5.2 HCV treatment for kidney transplant recipients

Kidney transplant recipients infected with HCV should be evaluated for treatment with a DAA-based regimen. The choice of DAA regimen should be based on HCV genotype and subtype, viral load, prior treatment history, drug interactions, GFR, stage of liver fibrosis, liver transplant candidacy, and comorbidities. Specific to drug–drug interactions, clinicians should do a pre-treatment assessment of the interaction between DAA and immunosuppressive drugs [8]. Drug interactions are an important issue in kidney transplant recipients, and DAA treatment could provoke the elevation or suppression of the immunosuppressive drug level in the blood, resulting in graft rejection or toxicity. Immunosuppressive drugs called calcineurin inhibitors (tacrolimus, cyclosporine), are metabolized by cytochrome P-450 [10]. In those receiving calcineurin inhibitors, monitoring of the calcineurin inhibitor level should be done [8]. Protease inhibitors DAA are associated with a significant risk of interaction with calcineurin inhibitors. Non-structural protein 5A/NS5A inhibitors (ledipasvir, daclastavir) and non-structural protein 5B/NS5B inhibitors (sofosbuvir) are associated with a lower risk of interaction with calcineurin inhibitors. Concurrent use of elbasvir-grasoprevir with cyclosporine is not recommended because it increases the area under the curve for grazoprevir by 15-fold and that for elbasvir by 2-fold. Grazoprevir/elbasvir could induce an elevation of tacrolimus levels by 43%. On the other hand, protease inhibitors do not interact with mycophenolate mofetil [10]. **Figure 1** shows the algorithm of DAA treatment in kidney transplant recipients.

According to one study, DAA treatment achieved SVR rates of more than 95% in kidney transplant recipients [8]. Evidence showed that glecaprevir/pibrentasvir treatment in kidney and liver transplant recipients could achieve an overall SVR of 98% [41]. For patients with GFR ≥30 mL/min/1.73 m^2, a sofosbuvir-based regimen has been added to the therapeutic options. A previous study discovered that kidney transplant recipients with GFR ≥ 40 mL/min/1.73 m^2 had a 100% SVR after receiving the sofosbuvir/ledipasvir regimen [42]. Lai and colleagues [42] reported that grazoprevir/elbasvir for 12 weeks is highly effective in genotype 1b HCV-infected liver and kidney transplant recipients [43].

5.3 Management of patients with HCV infection, before and after kidney transplantation

HCV infection in potential kidney transplant recipients can be treated before or after kidney transplantation [10, 44, 45]. The timing of DAA treatment depends on donor type, wait list times, HCV genotypes, transplantation center-specific policies,

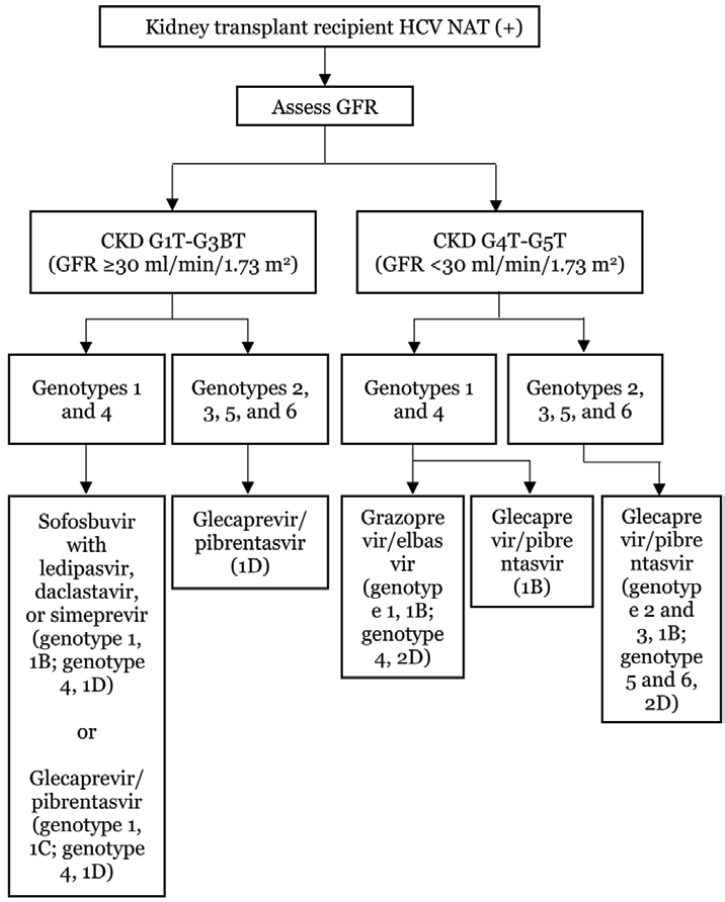

Figure 1.
Treatment scheme for kidney transplant recipients. Recommendation grades (1 or 2) and strength of evidence (A to D) are provided for each recommended regimen and HCV genotype. Sofosbuvir/velpatasvir-based regimens may be considered in kidney transplant recipients with GFR ≥ 30 mL/min/1.73 m² given their availability in certain jurisdictions. T suffix in GFR categories (e.g., G1T) denotes transplant recipient [8].

and the degree of liver fibrosis. For deceased donors, KDIGO recommends that kidney from HCV NAT-positive donors be directed to kidney transplant recipients who are NAT-positive. But, if acceptance of a graft from HCV NAT-positive donor could reduce the wait time for transplantation, patients could undergo transplantation with an HCV-positive kidney and get DAA treatment after transplantation [10, 44]. On the other side, HCV NAT-positive living donors should be treated and SVR should be confirmed before transplantation, as long as the patients have no evidence of cirrhosis [8, 46]. **Figure 2** shows the HCV treatment algorithm in kidney transplant candidates.

5.4 Sofosbuvir-based regimen in HCV-infected patients with CKD GFR category G4-G5D

Although alternative DAA (glecaprevir/pibrentasvir and grazoprevir-elbasvir) had already been approved for advanced CKD patients, a need for safety and efficacy data on sofosbuvir-based regimens remained, due to limited access to those alternative DAA in some countries and the risks associated with the use of protease-inhibitor containing

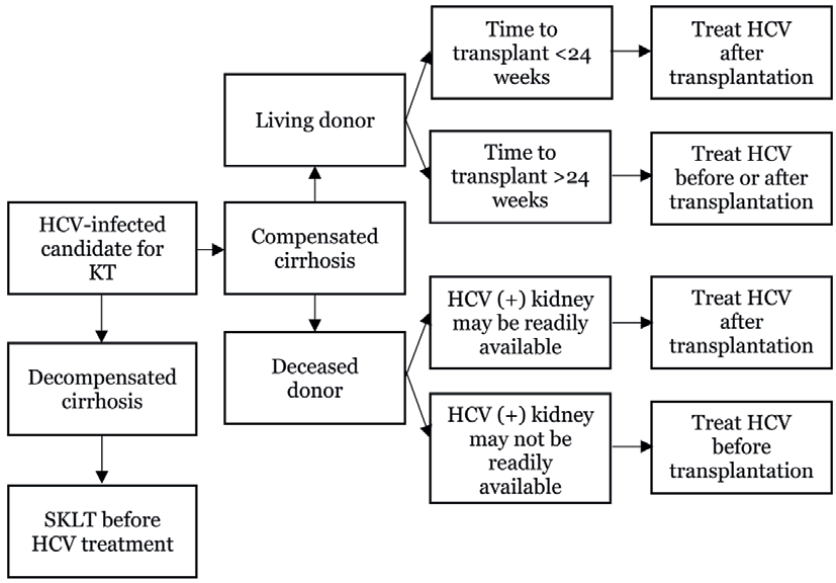

Figure 2.
HCV treatment algorithm for kidney transplant candidates. KT, kidney transplant; SKLT, simultaneous liver-kidney transplant [10].

regimens in patients with advanced fibrosis or cirrhosis. As a consequence of this phenomenon, sofosbuvir-based regimens were being administered off-label to patients with ESRD, including those undergoing HD. AASLD currently recommends all DAAs for GFR ≤ 30 mL/min [15]. US Food and Drug Administration (FDA) also approved the use of three sofosbuvir-based regimens (sofosbuvir/ledipasvir, sofosbuvir-velpatasvir, and sofosbuvir-velpatasvir-voxilaprevir) in patients with GFR below 30 mL/min [45].

Sofosbuvir, a non-structural N5B polymerase inhibitor, was approved in 2013 and is now the backbone of many DAA regimens, enhancing the cure bar above 90% [47–49]. Sofosbuvir has large renal excretion and has been initially licensed for patients with GFR of more than 30 mL/min [12–14]. According to the KDIGO guidelines, sofosbuvir-based regimen is not indicated on label in CKD patients with GFR ≤ 30 mL/min [44]. The circulating metabolite of sofosbuvir, GS-331007, is primarily eliminated by the kidney and accumulates up to 5- to 20-fold in patients with severe kidney impairment or ESRD, respectively [50]. It is still unclear about the association between the increased concentration of GS-331007 and renal toxicity. There were case reports that revealed acute interstitial nephritis in sofosbuvir/ledipasvir [51] and sofosbuvir/daclastavir [52] consumption, in the setting of CKD. Unfortunately, kidney injury has not been reported in a larger clinical trial of sofosbuvir [45].

Several studies have reported the efficacy and safety of sofosbuvir-based regimens in those with GFR ≤30 mL/min. A real-life multicenter retrospective cohort study on 4944 chronic HCV patients with CKD, reported that sofosbuvir-based regimens are effective and safe for treating patients with severe CKD and those with associated hepatic decompensation. In this study, SVR12 was achieved in 96.7% of patients with severe renal impairment [53].

A multicenter, prospective, single-center study of patients undergoing HD evaluated full-dose sofosbuvir once daily or three times weekly paired with simeprevir, daclastavir, ledipasvir, or ribavirin. A sofosbuvir-based regimen was administered

after HD, and they reported that GS-331007 did not accumulate between dialysis sessions or during therapy. SVR12 or SVR24 was achieved in 83% of subjects, and no serious adverse events (SAE) occurred (including cardiac events) [54].

An observational study in patients on HD in Rabat (Morocco) revealed a 100% SVR after being treated with sofosbuvir (400 mg) and daclastavir (60 mg), three times per week (after HD session). None of them had side effects or developed hepatobiliary and cardiac toxicity. There were fatigue and headache side effects, but the symptoms disappeared after the end of treatment [55].

In Jinnah Hospital in Lahore, Pakistan, a prospective open-label, parallel, non-randomized interventional trial was conducted in 36 patients with HCV on maintenance HD. The subjects were enrolled and then equally allocated in 1:1 ratio to group 1 who received 400 mg daily sofosbuvir/60 mg daily daclatasvir and group 2 who received three times per week of 400 mg sofosbuvir and daily 60 mg daclatasvir for 12 weeks. Patients with compensated liver cirrhosis got therapy for 24 weeks. They reported that sofosbuvir/declatsavir is highly effective and tolerable in patients with HCV genotype 1 & 3 undergoing HD, especially when given daily [56].

A phase II clinical trial (single-arm study) looked at the treatment response of 59 patients with genotype 1–6 HCV infection and ESRD who were on HD (92%) or PD (8%) at the time. All patients received sofosbuvir/velpatasvir (400/100 mg) once daily for 12 weeks. About 32% of patients had kidney transplant before and 29% of patients had liver cirrhosis. After 12 weeks, 56 of 59 patients achieved SVR (95%; 95% CI 86–99%). SAE was reported for 19% of patients, and all were deemed to be unrelated to the DAA regimen [57].

The efficacy and safety of sofosbuvir-based regimens in CKD patients with GFR category G4-G5D were also revealed by systematic review and meta-analysis. Li et al. [2] analyze 21 studies in which HCV patients with stage 4 or 5 CKD received sofosbuvir-based therapy. In total 717 patients were enrolled, including 58.4% HD patients or PD recipients. Pooled SVR12/24 was 97.1% and the SAE rate was 4.8%. There was no significant difference at SVR12/24 (97.1 vs. 96.2%, $p = 0.72$) or SAE rate (8.8 vs. 2.9%, $p = 0.13$) between subgroups applying the full or decreased dose of sofosbuvir. Patients with and without liver cirrhosis achieved comparable SVR (RR 0.93, 95% CI 0.85–1.02).

A systematic review and meta-analysis by Fabrizi et al. [12] showed that sofosbuvir-based regimens were safe and effective in patients with CKD stages 4–5. Thirty clinical studies were retrieved, then the pooled SVR12 and SAE rates were 0.99 (95% CI, 0.97;1.0, $I^2 = 99.8\%$) and 0.09 (95% CI, 0.05;0.13, $I^2 = 84.3\%$), respectively. The pooled drop-out rate due to adverse events was 0.02 (95% CI, −0.01;0.04, $I^2 = 16.1\%$). Reduced GFR was found in 14.19% of patients, and 26.38% of patients got anemia. SAE were common in full-dose sofosbuvir and ribavirin-based regimens.

Another systematic review and meta-analysis of 20 studies also suggested that sofosbuvir-based regimens for HD patients were effective and safe. The efficacy of the sofosbuvir-based regimen was 92% (95% CI 80–99%), 98% (95% CI 96–100%), and 100% (95% CI 95–100%) for the following doses: 400 mg on alternate days, 400 mg daily, and 200 mg daily, respectively. Among the studies that reported adverse events, anemia was the most common, with a pooled prevalence of 15% [58].

6. Conclusions

Effective detection, evaluation, and treatment for HCV infection can improve kidney and cardiovascular outcomes in CKD patients. Several studies have found an

association between SVR and a reduction in ESRD-related mortality, a reduction in vascular events, an improvement in cryoglobulinemic vasculitis, and a reduced risk of deterioration in kidney function. The development of DAAs enabled a greater rate of HCV eradication in CKD patients. Any licensed DAA regimen can be used in patients with CKD G1-G3b. The recommended DAA regimens for CKD G4–5D, are the sofosbuvir-free combination therapies (grazoprevir/elbasvir and glecaprevir/pibrentasvir). While KDIGO did not recommend sofosbuvir-based regimens as the drug of choice in patients with CKD G4–5D, data from multiple studies have demonstrated the effectiveness and safety of sofosbuvir-based regimens in CKD G4-G5D. Currently, AASLD and the US FDA have approved the use of sofosbuvir-based regimens in CKD G4-G5D patients.

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Appendices

GFR categories (mL/min/1.73 m²), description and range				Persistent albuminuria categories, description and range		
				A1	A2	A3
				Normal to mildly increased	Moderately increased	Severely increased
				<30 mg/g <3 mg/mmol	30–300 mg/g 3–30 mg/mmol	>300 mg/g >30 mg/mmol
	G1	Normal or high	≥90	Low risk	Moderately increased risk	High risk
	G2	Mildly decreased	60–89	Low risk	Moderately increased risk	High risk
	G3a	Mildly to moderately decreased	45–59	Moderately increased risk	High risk	Very high risk
	G3b	Moderately to severely decreased	30–44	High risk	Very high risk	Very high risk
	G4	Severely decreased	15–29	Very high risk	Very high risk	Very high risk
	G5	Kidney failure	<15	Very high risk	Very high risk	Very high risk

Figure A1.
Current CKD classification used by KDIGO guideline [10].

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Chapter 7

Risk Factors Associated with Development of Hepatocellular Carcinoma in Hepatitis C Virus Patients

Reem El-Shenawy, Sally Farouk, Naiera Helmy and Noha Bader El Din

Abstract

Hepatitis C virus (HCV) is the main etiology of advanced liver fibrosis and cirrhosis with significant risk of progression to hepatocellular carcinoma (HCC). Several epidemiologic studies have documented a lot of risk factors related to the progression of HCC in chronic HCV patients. Factors that increase the risk of HCC development include obesity, diabetes mellitus, nonalcoholic fatty liver disease, aflatoxin exposure, alcohol consumption, occult hepatitis C infection, and genetic variations. HCV patients with genotypes 3 and 1 are also more liable to develop HCC. Also, male gender and higher age are considered as independent risk factors for HCC. Using the newly discovered direct-acting antivirals (DAAs), great improvement in sustained virological immune response (SVR) has occurred >90% in treated patients irrespective of their fibrosis level. Nevertheless, the progression to HCC in HCV patients who achieve SVR stays vulnerable to HCC development, especially patients with advanced fibrosis and/or cirrhosis.

Keywords: HCC, pro-inflammatory cytokines, oxidative stress, SNPs, DAAs, apoptosis

1. Introduction

Infection with hepatitis C virus (HCV) is a worldwide major health problem, where its prevalence had an assessed 2.8% elevate through the last decade, to more than 185 million infections (3% of the world's population) [1]. Chronic HCV infection is a principal cause of end-stage liver disease, HCC, and liver-related deaths. HCV has seven genotypes (gt 1–7) and about 100 subtypes. The infection rate and subtype predominance are country dependent. The infection rate variance between low and high-endemic countries is about 20% [2]. The lowest prevalence of HCV occurs in Australia, North America, North, and Western Europe. On the other hand, the highest prevalence of HCV infection occurs in African and Asian countries, where three-quarters of infected individuals are living in middle-income

countries, including India, Nigeria, Pakistan, Egypt, China, and Russia together accounting for more than half of world HCV infection [3]. The highest virus prevalence was recorded in Egypt, due to community-wide mass anti-schistosomiasis treatment from the 1950s to the 1980s. At that time tartar emetic injections were the standard treatment therapy [4], and 22% of population were infected. Treatment especially targeted children and young adults, and more than two million shots were given yearly to almost 250,000 patients. Each patient was assumed to have a series of injections with the average number of injections per patient being nine in the 1960s, which then declined to six after 1975. HCV infection has a special situation in Egypt early in its history and will continue till elimination, hopefully, in the near future [1].

2. Mechanisms of HCV-related carcinogenesis

Chronic hepatitis and cirrhosis perform major risk factors for HCC development. Hepatic carcinogenesis is a multifactorial process that takes years, including chemical exposure or viral agents causing inflammatory reactions leading to mitochondrial oxidative damage, cytokine response, necrosis of hepatocytes, and finally malignant transformation and clonal expression. Hepatocytes become malignant through elevated liver cell turnover, due to chronic liver injury and renewal, during inflammation and oxidative stress states. Viral proteins may upregulate mitogenic pathways to prevent apoptosis and persuade the production of reactive oxygen species (ROS) [5]. Moreover, the virus prompts continuous inflammation with liver-infiltrating lymphocytes and cytokines, such as lymphotoxin (LTα and LTβ), that are closely related to development of HCC. Chronic inflammation aggravates ROS production which is a fundamental cause of genetic mutations. ROS are also correlated to inducing the transforming growth factor (TGF-β) pathway, activating hepatic stellate cell and fibrogenesis. Together, TGF-β and Toll-like receptor (TLR4) plays a vital role in the epithelial–mesenchymal transition. HCV dysregulates the patient's lipid metabolism leading to the accumulation of liver fat that is highly correlated to HCC development. HCV also persuades angiogenic and metastatic pathways (**Figure 1**) [6].

3. Risk factors for HCC development in HCV patients

HCC etiology is generally supposed to be related to liver cirrhosis, viral hepatitis, alcoholic liver disease, metabolic-related fatty liver disease, and aflatoxin infection. Among them, viral hepatitis is the most important factor, of which chronic hepatitis B (HBV) and HCV infections are the most common [7].

3.1 Age and gender

Age and gender are independent risk factors for HCC patients with HCV infection. HCC represents the third most popular malignancy among men and 7th among women worldwide [8]. Men have a greater threat of HCC than women due to their exposure to other risk factors such as alcohol abuse, smoking plus persistent HCV or HBV infection [9]. Otherwise, sex hormones and pregnancy in women patients have a role in infection with HCV and its progression to HCC [10]. The age-standardized

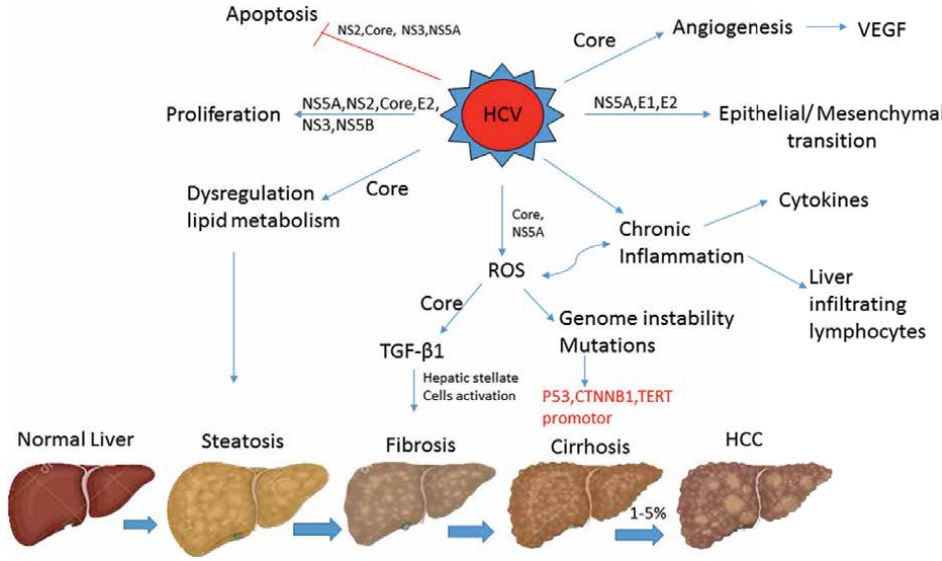

Figure 1.
HCV-related mechanisms of carcinogenesis.

incidence rate (ASIR) in Eastern Mediterranean countries stated that the incidence of HCC in men was 8.1/100000 while in women was 4.7/100000. The occurrence of HCC is low before age 40 but then elevates exponentially. HCC investigation is recommended for Asian men greater than 40 years of age and Asian women greater than 50 years. The guidelines also suggest that surveillance should begin earlier at a younger age for African/North American blacks, without any precise specification of the age cut-off year [11]. Also, male patients who are older than 40 years and have elevated levels of alanine aminotransferase might benefit from HCC surveillance, irrespective of race [12].

3.2 Alcohol abuse

HCV infection rates have been shown to be significantly higher in alcoholic patients than in nonalcoholic ones [8]. Furthermore, alcohol consumption in chronic HCV patients has been associated with an accelerated rate of fibrosis and a higher risk of development of liver cirrhosis and eventually HCC [13]. Excessive alcohol consumption has been considered as the main reason for high HCC rates in various regions like Central and North Europe, while low alcohol consumption has been linked to low HCC mortality rates in countries like France [14]. Excessive alcohol intake leads to hepatocarcinogenesis due to the high production of acetaldehyde, which is mutagenic metabolite of ethanol. Additionally, it increases oxidative stress, DNA damage and causes a carcinogenic tissue microenvironment that might have a synergistic effect with viral hepatitis and metabolic syndrome [15].

HCC development might also be a direct outcome of increased HCV replication and weakening of the interferon's antiviral activity resulting from alcohol consumption. Finally, weakened cellular immunity as a result of dendritic cell dysfunction, in addition to high oxidative stress and mitochondrial injury owing to alcohol consumption, all together confer to HCC progression [16].

3.3 Diabetes mellitus and non-alcoholic fatty liver disease

HCV-infected individuals who are obese, have diabetes mellitus (DM), and/or have nonalcoholic fatty liver disease (NAFLD) are more likely to develop HCC [8]. Around 25% of the global population are affected by NAFLD, among those 60% are affected by non-alcoholic steatohepatitis (NASH), evolving HCC at a rate of 5.29/1000 person per year [14]. Definitely, HCV patients in the US have been found to develop HCC more quickly than patients in China and fatty liver disease was reported to be a main provider of this difference [17]. According to epidemiological studies, DM has been associated with a two- or threefold rise in risk of HCC development in individuals with chronic HCV infection [18]. Type 2 diabetes mellitus (T2DM) can be linked to central obesity that induces carcinogenesis through the several mechanisms shown in **Figure 2**.

T2DM and NAFLD have been reported to be strongly associated with high hepatic/peripheral insulin resistance, and lipotoxicity, causing high secretion of various pro-inflammatory cytokines (e.g., C-reactive protein, interleukin-1, interleukin-6, tumor necrosis factor-alpha, tumor growth factor-beta), vasoactive factors and pro-oxidant molecules into bloodstream [19]. Many studies verified that all these factors with increased insulin-like growth factor-1 (IGF-1) production driver may participate in HCC development by induction of hepatocellular growth/proliferation and by inhibiting cellular apoptosis in liver [20]. Several studies reported that when hepatocytes become steatotic, they become capable of producing ROS causing cytotoxicity and DNA damage which in turn cause development of HCC [19].

Recently, accumulated data introduced that alterations in gut microbiota might have a role in obesity, T2DM pathogenesis, and NAFLD, which is implicated in hepatic carcinogenesis [21].

3.4 HCV genotypes

A main peculiarity of HCV is its high degree of heterogeneity. Recently, HCV has been classified into seven genotypes based on the sequence of the viral genome [22]. The rate of HCC development from chronic hepatitis varies and might be associated with multiple factors, such as old age, long duration of infection, gender or alcohol consumption >50 g/day, as well as viral factors like viral genotype/subtype or viral load [23].

Interestingly, patients infected with HCV genotype 3 are more likely to develop end-stage liver diseases and HCC, and eventually liver-related death than other genotypes [24]. However, even after eradication of the disease, the probability of developing HCC is still high. It has been proposed that this specific genotype undergoes a particular oncogenic mechanism that drives to HCC, even if patients are non-cirrhotic [25]. Likewise, HCV genotype 1b specifically may have a pivotal role in HCC progression, especially in patients with early-stage liver disease [26]. Therefore, combining genotypes 1 and 3 and the fast progression of liver damage might lead to low survival rates in HCV-related HCC patients [22]. Moreover, genotype 6 which is mainly prevalent in Southern China and Southeast Asian countries, including Vietnam, Malaysia, Cambodia, and Thailand, showed a higher risk of developing HCC among cirrhotic patients [27].

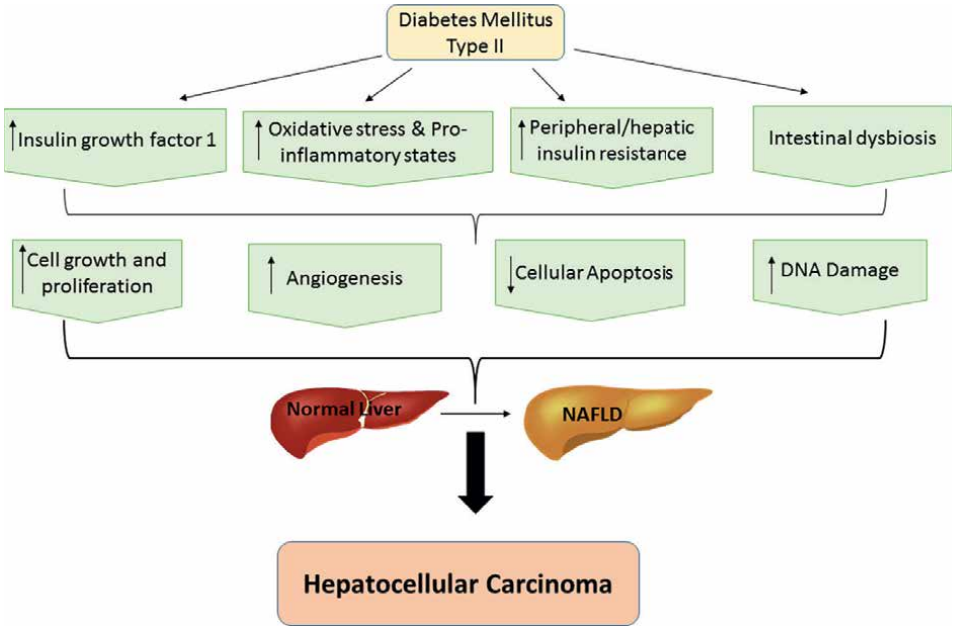

Figure 2.
Biological mechanisms linking type 2 diabetes mellitus and NAFLD to the development of HCC.

3.5 Strength of immune system

The immune system includes a wide variety of cells and mediators that interact in a sophisticated and dynamic network to confer protection against foreign pathogens while concurrently maintaining tolerance toward self-antigens [14]. One of the immune system components that elicited against foreign pathogens is "cytokines" which stimulate a host response aimed at controlling cellular stress and reducing cellular damage [28]. But, when host response failed to repair the injury, it stimulates excessive induction of immune cell infiltration leading to alteration and continual cytokine production which can impact several stages of cancer progression and development [29].

The HCV-mediated cellular immune response is generally weak, where the immune reactivity observed in HCV-infected patients' livers is largely unspecific. Conversely, innate immune cells are thought to play a key role in HCV immunopathology [30]. Intra-hepatic production of cytokines and chemokines induced by HCV infection leads to the recruitment of non-specific lymphocytes. This pathway induces itself in the absence of viral clearance, causing necro-inflammatory and fibrotic liver disease. A significant lymphocyte infiltration was observed in the portal tracts in liver of HCV P21 core transgenic mice and was associated with elevated serum alanine transaminase (ALT) levels [31]. These changes in liver function are related to the HCC progression and development. Several examples of cytokine have been identified as indicator of elevated HCC risk as shown in **Figure 3**.

Chronic hepatitis C is characterized by persistent hepatic inflammation. This is demonstrated by a higher production of pro-inflammatory cytokines and chemokines, which basically initiates from infected hepatocytes, circulating leukocytes, or Kupffer cells. These cells produce cytokines as response to cellular signaling cascades

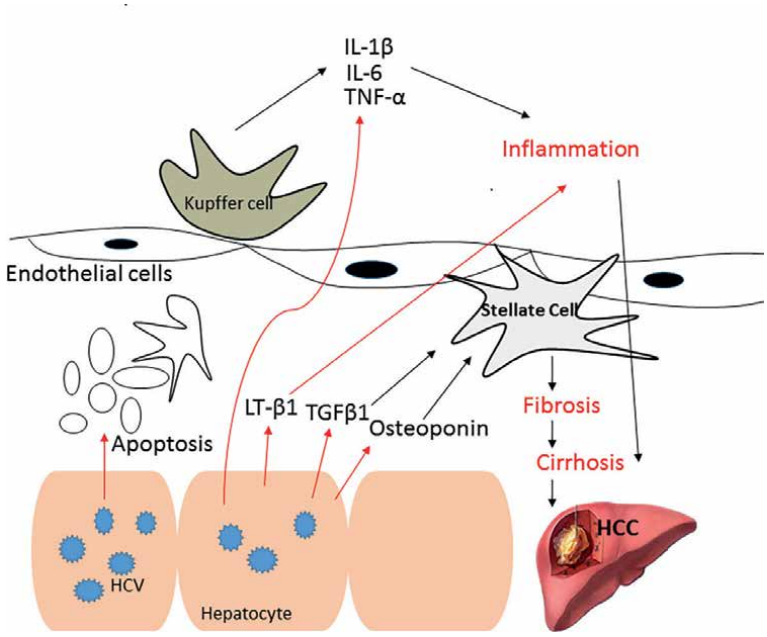

Figure 3.
Pro-inflammatory cytokines during HCV-related HCC.

activation, virus-induced oxidative stress, apoptosis of infected cells, or direct activation of innate and adaptive immunity. Among these cytokines are interleukins 1β (IL-1β), 6 (IL-6), 8 (IL-8), and tumor necrosis factor α (TNF-α), in addition to lymphotoxin (LT).

3.6 Single nucleotide polymorphisms (SNPs)

Genetic alterations like SNPs, might change the disease risk and hence may be applied as predictive markers of the disease outcome. To that end, SNPs are useful indicators of the increased risk of HCC [8]. Accumulated data suggest a relationship between SNPs of certain genes and the vulnerability to HCC. For instance, the tolloid–like 1 gene (TLL1) on chromosome 4. A strong relation between TLL1 and HCC development in chronic HCV patients who achieved SVR after treatment was reported [28]. Furthermore, a transformation suppressor gene; the reversion-inducing-cysteine-rich protein with kazal motifs (RECK) gene polymorphism (rs11788747) has been shown to be involved in the pathogenesis of HCC and malignancies [32]. Nevertheless, a recent study performed on a group of Egyptian patients suggested that the RECK gene rs10814325 TT genotype might rather be linked to disease progression and metastasis than being a risk factor for HCC development in HCV patients [33].

Moreover, matrix metalloproteinases (MMPs) play an important role in tissue remodeling during the process of embryogenesis and tissue evolution as well as in wound healing in normal physiologic conditions [34]. Different variants of the MMP-11 gene could be related to clinical status and HCC progression. Subjects carrying the CT + TT allele of the MMP-11 SNP (rs738791) showed a higher risk of HCC development than wild-type (C/C) carriers. Thus, genetic variability in the MMP-11

gene represents a substantial predictor of early stages of HCC onset and a reliable biomarker for disease progression [35].

Additionally, IL-28B (rs12979860) TT genotype has been found to be more dominant in patients with progressive fibrosis, hepatic cirrhosis, HCC, and in the course of HCV recurrence after liver transplantation (LT) [36]. Hence, it seems to be associated with minor consequences in HCV chronic patients and increases the risk of HCC development. The T allele might be considered as a genetic risk factor for HCV-related carcinogenesis, antiviral therapy failure, and post-transplant fibrosis progression [37].

Likewise, transforming growth factor (TGF)-β is a multifunctional profibrotic cytokine that is found in three isoforms. There is a significant association between TGF-β1 869C/T and -509C/T polymorphisms and HCC risk [38]. It is important to note that TGF-β is considered a central mediator of fibrogenesis and has a vital role in the regulation of tumorigenesis [39]. Also, tumor necrosis factor-alpha (TNF-α), which is a potent antiviral cytokine possessing a broad spectrum of proinflammatory activities, plays a crucial role in host immunity against HCV infection, as cytotoxic T lymphocytes (CTLs) in the liver have been shown to secrete TNF-α [40]. Interestingly, HCV infection has been associated with high levels of circulating TNF-α and HCV infection stimulates the production of TNF-α in human hepatocytes. Thus, increased levels of TNF-α are correlated with the severity of tissue injury, hepatic inflammation, fibrosis, and eventually HCC [41]. Another SNP is major histocompatibility complex class I chain-related gene A (MICA) which plays a vital role in immune activation and surveillance against infection and tumorigenesis, and MICASNP rs2596542G > A is associated with HCC development among the Asian, Caucasian, and African ethnicity in certain genetic models [42].

3.7 Viral infection

3.7.1 Hepatitis B virus (HBV)

The leading cause of HCC in Southeast Asia and sub-Saharan Africa is chronic HBV infection, although the percentage of HBV-induced HCC is dropping [43]. Countries, in which HBV occurrence is higher than 2%, show raised mortality rates due to HCC. HBV chronically infected subjects are at high risk of developing HCC that might reach up to 30-fold. This is due to the caused liver inflammation and damage in addition to epigenetic defects leading to HCC progression [44]. The occurrence of HCC is higher in HBV-HCV co-infection than in HBV or HCV monoinfection. Factors, such as long disease duration, increased fibrosis levels, intolerance of carbohydrates as well as higher HCV RNA levels, increase the risk of HCC development [45].

In a recent study, the incidence of HCC in patients coinfected with HCV/HBV was 6.4 per 100 person/year, while monoinfection with either HBV or HCV was 2.0 and 3.7, respectively. Additionally, risk of progression to HCC after 10 years of infection was raised to 45% in case of HBV/HCV coinfection compared to 16 and 28% in case of HBV and HCV monoinfection, respectively. Multiple studies also suggested a synergistic effect of both viruses in case of HBV/HCV coinfection [44]. Due to the weak immune responses toward hepatitis viruses and their failure to completely inhibit or eradicate the virus, this causes persistent stimulation of antigen-specific immune response in chronic patients. The ongoing secretion of cytokines and recruitment

of lymphocytes to the liver influences several cellular pathways leading to fibrosis, cirrhosis and eventually HCC [46].

3.7.2 Human immunodeficiency virus (HIV)

There is an increased rate of progression to cirrhosis and HCC in HIV/HCV co-infected patients [47]. One-third of HIV-infected patients have been reported to be coinfected with HCV, having an undesirable effect on HCV pathogenesis [48]. The percentage of liver fibrosis was reported as up to 3 times higher in cases of HIV/HCV coinfection than in HCV monoinfection. HIV also plays a role similar to HBV co-infection. Many studies depicted that 75% of HCV-related mortalities happen between the age of 45 and 65 years, especially in subjects co-infected with HIV or HBV and suffering from hepatic complications such as cirrhosis and HCC [49]. HCV has a necrotic effect on hepatocytes. HIV was found to speed up liver disease progression in HCV patients, as HCV replication augments HIV's presence leading to high levels of HCV RNA. Furthermore, HIV has a destructive role on the immune system causing the exhaustion of CD4-T cells which cause a high diminution of CD4 cells, and the reduction of CD4 cell stimulates cirrhosis and HCC development [50].

3.7.3 Human cytomegalovirus (HCMV)

Human cytomegalovirus (HCMV) is a herpesvirus-specific species that infect a major part of the population in world and causes asymptomatic latent infection in healthy subjects [51]. However, it can cause severe disease in absence of a robust immune response which remains an important reason for morbidity in immune-compromised individuals where it may clear as symptomatic as hepatitis disease [52]. Most organs and tissues of the human body can be infected by HCMV, such as hepatocytes, fibroblasts, endothelial and neuronal cells, besides blood monocytes and macrophages [53]. HCC patients have a higher significant prevalence of HCMV than patients without HCC, HCMV is positively associated with serum IL-6 levels in cirrhotic patients, and is positively related to the presence of other hepatotropic viruses, such as HCV and HBV [54]. Higher incidence of HCMV among HCV genotype 4 infected patients with less response to IFN therapy has been reported in previous reports [55]. Most of previous reports on HCMV pathogenicity introduced some HCMV proteins as fibrogenesis inducers, such as human (CMV IE1 or IE2). Besides, several CMV proteins modulate the cellular apoptotic machinery like CMV UL97, CMV UL36, and CMV IE86 [54]. An important study on Egyptian patients with genotype 4 documented the dysregulation of the anti-fibrotic pathway (i.e., dysregulation of the JAK–STAT pathway). This dysregulation is likely to be a key molecular and immunological factor for increased susceptibility to HCC development in HCV/CMV co-infected patients with advanced-stage of liver fibrosis [55].

3.8 Effect of HCV treatment

Globally, HCC is the third important reason for cancer-related death, with more than half a million patients, being affected annually. Cirrhosis is the predominate risk factor for HCC, as the accumulative risk for HCC development ranges from 5 to 30% within 5 years among patients with cirrhosis [14], whereas the risk of HCC development in HCV-related cirrhotic patients is about 2–8% annually. Patients with

established fibrosis are at higher risk of HCC development HCC than patients with a lower fibrosis level [56].

3.9 Treatment with interferon-based regimens

In the past decades, chronic hepatitis C patients were treated with IFN–based regimens for more than 20 years. Many studies have confirmed the long-term effects of this treatment protocol in achieving an SVR and curing about 50% of treated patients [57]. According to several studies, patients who achieved an SVR after IFN-α treatment, showed improved liver fibrosis, a lower risk of infection-related complications, and an overall reduced risk of de novo HCC development and mortality rates compared to those who failed to achieve SVR [58].

However, the remarkable benefits of this treatment protocol did not reduce HCC incidence, and the risk of developing HCC is not totally eliminated in patients with severe fibrosis or cirrhosis. Such conditions arise with an incidence of 0.3–4% [59].

In a Japanese study, two groups of patients were enrolled for treatment with IFN-α and ribavirin combination; a total of 863 non-cirrhotic patients and 150 cirrhotic patients developed HCC after a median 3.6-year follow-up period. In the non-cirrhotic group, the accumulative incidence rate of HCC development in the SVR group (1.7%) was significantly lower ($P = 0.003$) than in the non-responder group (7.6%). On the other hand, the accumulative incidence rates of HCC for the SVR group (18.9%) in the cirrhotic patients' group were also significantly lower ($P = 0.03$) than in the non-responder group (39.4%) [60].

The key predictors of HCC have been found to include age (more than 60 years old); male sex; platelet count (below 150×10^9/L), levels of AFP (higher than 10 ng/mL); liver cirrhosis, and failure to achieve SVR [61]. In a study conducted by El-Seraget *et al.*, the HCC risk after achieving SVR was reported to be 0.33% annually. Also, the annual HCC risk remained high among cirrhotic patients at the time of treatment (1.39%) and in cured patients older than age 64 (0.95%) with or without cirrhosis [62]. These outcomes recommend HCV treatment in early stage before the development of cirrhosis besides long-term monitoring of HCC in cirrhotic patients even after reaching the SVR [63].

3.9.1 Treatment with DAAs

3.9.1.1 Immunological mechanisms implicated in HCC development with DAA

The exact reasons for high rates of tumor development or relapse after the DAAs treatment protocol remains unknown. However, there is one hypothesis that suggests that the anti-tumor host response might be dysregulated after the swift reduction of HCV viral load generated by DAAs, which may promote tumor recurrence [64]. In addition, the growth of existing precancerous lesions is due to immune distortion. HCV infection is activating the intra-hepatic cellular immune response, triggering the elevation of IFN-stimulated gene expression as well as stimulating the natural killer (NK) cells. Other studies suggested that DAA-mediated HCV clearance is associated with the intra-hepatic immune activation loss confirmed by reduced CXCL10 and CXCL11 chemokines levels, and normalization of NK cells phenotype and function. In the same context, Debes *et al*. has recognized a group of twelve immune mediators containing cytokines; growth factors and apoptosis markers elevated in the serum of patients that developed de novo or recurrent HCC before initiation of DAA treatment

compared to treated patients who did not develop HCC [65]. These results suggest that patients with HCC history may previously express a different form of immune mediators before DAAs treatment (**Figure 4**) [66].

3.9.1.2 Risk of de novo HCC

DAAs treatment has proved to be successfully lowering the incidence of viral hepatitis worldwide, achieving SVR in more than 90% of patients enrolled for treatment regardless of the level of liver fibrosis [61]. There were several studies on the development of HCC following DAAs treatment (**Table 1**) [24, 67–76].

Undoubtedly, the HCV-treated population has significantly changed in the DAAs era, where it currently includes numerous patients with different HCC risk factors. This might explain the reason why the newer cohorts of patients treated with DAAs may encounter whole new HCC risks compared to those expected based on their medical history. HCV cirrhotic patients who reach SVR with DAAs treatment remain at high HCC risk, and for that, they should continue a long-run HCC screening.

3.9.1.3 Risk of HCC recurrence

A controversial issue is the impact of a DAAs-based SVR on the risks of HCC recurrence after early HCC treatment. In this area, several study cohorts have investigated the risk of HCC recurrence after DAAs treatment protocol as shown in **Table 2** [59, 74, 76–79].

Well-designed studies with potent comparison arms are needed for solid assessment of DAAs impact on HCC recurrence. Presently, HCV-cirrhotic patients who underwent ablation or resection of HCC should not be dissuaded from receiving DAAs treatment aiming to restrain the liver disease progression. Otherwise, rigorous screening is a must to confirm tumor clearance in HCC patients prior to the initiation of DAAs treatment protocol. Also, monitoring HCC by liver imaging alongside AFP testing should be open-endedly continued at least twice a year after reaching SVR.

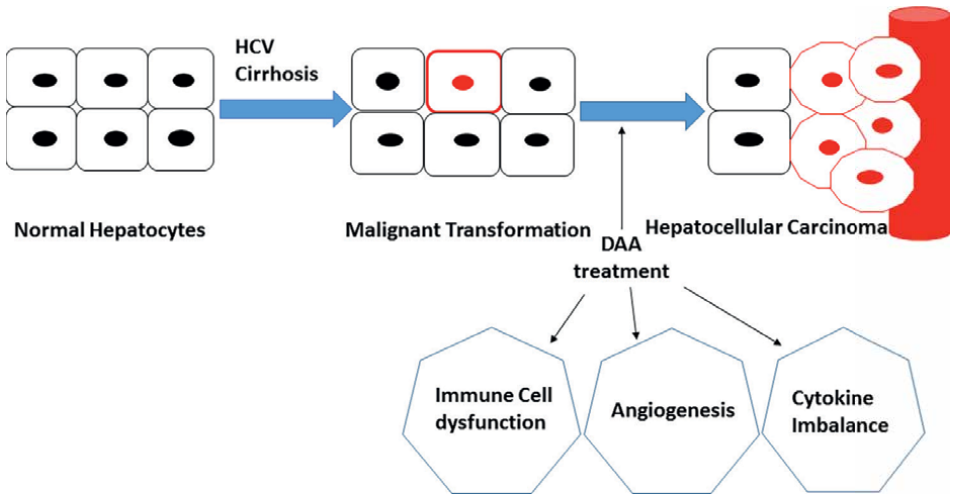

Figure 4.
Mechanisms involved in HCC recurrence after DAAs treatment for HCV chronic patients.

Authors (reference)	Type of study	n	Cirrhosis (%)	Follow-up (Medium)	HCC incidence (%)
Cardoso [66]	Retrospective	54	100	12 months after SVR	7.4
Kozbial [67]	Retrospective	195	NA	13 months after DAA cessation	6.6
Ravi [68]	Retrospective	66	100	6 months after DAA cessation	9.1
Foster [69]	Prospective	467	77.5	6 months after DAA initiation	5.4
Conti [70]	Retrospective	285	100	6 months after DAA cessation	3.16
Cheung [71]	Prospective	406	100	15 months after DAA initiation	4 at 6 months (same as in 261 patients not receiving DAA) 6.7% at 1 year
Calleja [72]	Retrospective	3233	52	6 months after DAA initiation	0.9
Kobayashi [73]	Retrospective	77	NA	4 years	2.6
Toyoda [74]	NA	413	NA	NA	Annual incidence 0.62–0.85
Kanwal [24]	Retrospective	22,500	39	22,963 person-years after DAA cessation	SVR: 0.9 per 100 patient-years Non-SVR: 3.45 per 100 patient-years
Loannou [75]	Retrospective	21,948	24	6.1 years	1.32 per 100 patient-years

DAA, Direct Acting Antivirals, NA, Not Available, SVR, Sustained Virological Response, HCC, Hepatocellular Carcinoma.

Table 1.
Studies assessing de novo HCC risk after DAA therapy.

3.9.2 Occult HCV infection (OCI)

Occult HCV infection is defined as the persistence of viral RNA whether in liver cells or peripheral blood mononuclear cells (PBMCs) with no detectable HCV RNA levels in serum [80]. Recently, it was found that there is a markedly high prevalence of OCI-HCV following the DAAs treatment [61]. In spite of successful viral clearance, numerous patients continue to exhibit HCV-related disease progression [81]. Also, the risk of liver cancer development is not totally diminished, even in efficiently treated cirrhotic patients who reached the SVR. Although IFN-free treatment regimens are able to eradicate HCV infection more effectively, the replication of neoplastic clones continues in a setting of reduced inflammation because DAAs can eliminate HCV from serum rather than from cells. So, dual testing for HCV RNA in both serum and PBMCs is recommended at the end of HCV infection treatment with DDAs and during validation of the SVR after the initial response [82].

Authors reference	Type of study	n	Treatment for previous HCC	Follow-up (Medium)	HCC recurrence (%)
Reig [58]	Retrospective	58	Resection, ablation, TCE	6 months from DAA initiation	27.6 medium 3.5 months from DAA initiation to HCC recurrence
Conti [70]	Retrospective	59	Resection, ablation, TACE	12 months from HCC treatment to DAA initiation	28.8 within 24 weeks from DAA completion
Cabbibo [77]	Prospective	143	Resection, ablation, TACE	2 months from HCC treatment to DAA initiation Follow-up 9 months after DAA initiation	12 with 6 months of DAA initiation 26.6 with 12 months of DAA initiation
Calleja [72]	Retrospective	70	NA	20 months (mean) from HCC treatment	12.9 with 6 months of DAA initiation30 with 12 months of DAA initiation
Minami [78]	NA	27/926 (3%)	Ablation	16 months from DAA initiation	29.8 with 12 months of DAA initiation (vs 31 in untreated patients)

Table 2.
Study cohorts investigating HCC recurrence risk after DAAs treatment protocol.

3.10 Aflatoxin B1 (AFB1)

Aflatoxins are categorized into four types: B1, B2, G1, and G2; all known to be carcinogenic to both humans and animals. Among those, aflatoxin B1 is a known human carcinogen that has been shown to be a causal agent in the pathogenesis of HCC [83]. Aflatoxin is considered a food contaminant produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*, which develop under special environmental conditions characterized by high temperature and moisture, which is widely common in areas of Southeast Asia and Sub-Saharan Africa under 40 southern and 40 northern equator [84]. Aflatoxin can affect a wide range of food commodities, such as corns, peanuts, spices, maize, rice, and legumes as well as meat, milk, and dried fruits. Additionally, the exposure to aflatoxin can accelerate HBV and HCV-associated carcinogenesis by introducing mutations [85]. Once AFB1 is ingested, it is metabolized by the cytochrome P-450 system into the intermediate reactive oxygen species (ROS): afatoxin-8, 9-expoxide, which can interfere with DNA forming. AFB1-guanine products can react with proteins forming AFB1-albumin which ultimately leads to acute toxicity (aflatoxicosis) and lesions, respectively [86]. Failing to repair these lesions triggers DNA mutations in key genes; particularly "G to T" transversions. In this domain, the mutation (AGG to AGT, R249S) has been recognized at codon 249 in the TP53 tumor suppressor gene, specific for exposure to aflatoxin other studies have confirmed that this hotspot in TP53 is a special site for AFB1 adducts formation. Consequently, the formation of AFB1-DNA adducts over time rises the HCC risk (**Figure 5**) [87].

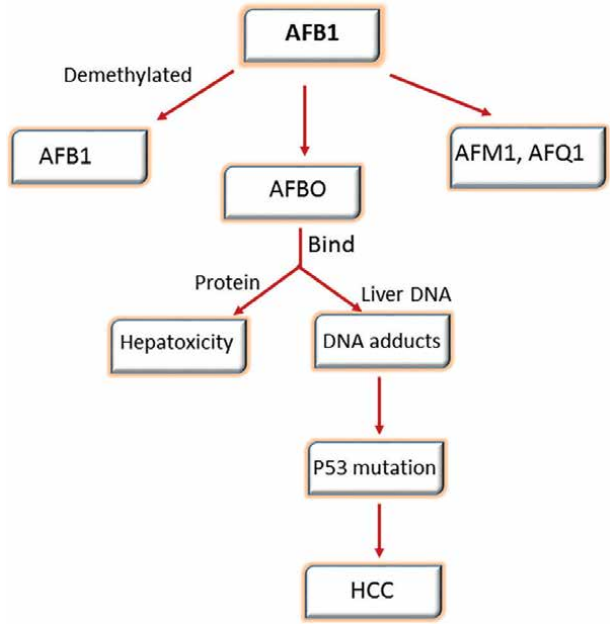

Figure 5.
Biotransformation of AFB1.

Conflict of interest

All authors declare that there is no conflict of interest.

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Chapter 8

Association between Hepatitis C Virus and Extrahepatic Tumors

Di Sun, Min Ding, Mengfan Ruan, Li Yang and Xingshun Qi

Abstract

Hepatitis C virus (HCV), an oncogenic virus, is a well-known risk factor for hepatocellular carcinoma. Some studies have shown an increased risk of extrahepatic tumors in HCV patients, but the risk of different types of extrahepatic tumors remains controversial. Early prevention of extrahepatic tumors in HCV patients should be further explored. Therefore, this chapter aims to explore the association between HCV infection and extrahepatic tumors.

Keywords: hepatitis C virus, extrahepatic tumors, lymphoma, breast cancer, pancreatic cancer, gastric cancer, cholangiocarcinoma, thyroid cancer, kidney cancer

1. Introduction

Viral infections are closely related to cancers. The International Agency for Research on Cancer (IARC) estimates that about one in five cancer cases worldwide are caused by infections, most of which are caused by viruses, including hepatitis C virus (HCV) [1]. HCV is an RNA hepatotropic and lymphotropic virus that infects about 180 million people worldwide [2]. Compared with chronic hepatitis B virus (HBV), chronic HCV infection typically leads to liver fibrosis and cirrhosis, which leads to a higher risk of liver cancer and mortality [3, 4]. In addition, it has been shown that HCV may infect organs and tissues other than the liver, such as peripheral blood cells (i.e., neutrophils, T cells, and B cells), kidney, skin, oral mucosa, pancreas, heart, gallbladder, intestinal tract, and adrenal gland, where HCV can be detected as well as its associated antigens, genome and/or replicative sequences [5]. In recent years, epidemiological studies have found that HCV infection is closely related to the occurrence of extrahepatic tumors [6]. Therefore, this chapter summarizes the association between HCV infection and the risk of several extrahepatic tumors.

2. HCV infection and lymphoma

Lymphoma is a group of malignant neoplasms of lymphocytes that can be classified into non-Hodgkin lymphoma (NHL) and Hodgkin lymphoma (HL) [7]. Lymphoma is associated with multiple factors, such as genetics, environment, and infection [8].

NHL is a hematologic malignancy with the highest prevalence worldwide, and its etiology is still unclear [9]. The relationship between HCV infection and NHL was first explored in 1994 by Ferri et al. [10]. The odds of HCV infection were higher in patients with NHL (34%) than those with HL (3%) and the healthy population (1.3%). A large number of studies have reported the relationship between HCV infection and NHL. The conclusions are mostly consistent in that patients with HCV are at a higher risk of developing NHL [11, 12]. For example, Zhu et al. conducted a meta-analysis, including 18 studies, during a period from 1999 to 2017 to explore the relationship between NHL and HCV [13]. They found that the risk of NHL was 69% higher among individuals with HCV infection compared to uninfected individuals. However, this study was mainly based on the European and American populations and ignored the impact of other factors, such as diet and living habits (e.g., high-fat diet, etc.), on the increased risk of NHL. In addition, Dal Maso et al. completed a meta-analysis of HCV infection and NHL based on 15 case–control studies and 3 prospective studies [14]. The etiologic fraction of NHL attributable to HCV varies greatly by country and may be up to 10% in areas where the HCV prevalence is high. By comparison, the studies included in this meta-analysis had several advantages. First, the studies included populations from the United States, Egypt, Italy, Japan, and Australia, and analyzed the differences among ethnicities. Second, some risk factors were adjusted, such as gender and age. Third, the diagnostic criteria of HCV infection were unified. In Southern and Eastern Europe, Japan, and the Southern United States, NHL and HCV have been reported to be highly correlated. There is no literature regarding the association of HCV infection with NHL in Central and Northern Europe, Canada, the Northern United States, and some Asian countries [15].

There are some potential mechanisms of HCV infection, leading to NHL. First, antiviral treatment appears to be effective in eliminating the clonal proliferation of B cells in patients with chronic HCV infection and may prevent the subsequent development of lymphoma [12]. Second, active replication of HCV in B cells may impair the cell cycle and mediate lymphomagenesis through the expression of HCV-related proteins, such as HCV core protein or nonstructural protein 3 (NS3). HCV enters B cells through the CD81 receptor, and the expression of viral core protein and NS3 in B cells leads to oxidative stress, which may eventually cause mutations and defective DNA repair [16]. Third, the E2 protein of HCV binds to the CD81 receptor and B-cell receptor (BCR) on B cells, and the complex formed after binding lowers the activation threshold of B cells, thereby promoting autoantibody production, which in turn leads to HCV-associated cryoglobulinemia. It is one of the extrahepatic manifestations caused by HCV infection, which can eventually progress to NHL [2, 17].

As for the association between HCV infection and HL, Franceschi et al. conducted a large prospective cohort study in 2011 and showed that the proportion of HCV infection in patients with HL was not significantly different from the general population [18]. Similarly, a meta-analysis conducted by Mullen et al. did not find any significant association between HCV infection and HL [19]. Therefore, further studies are needed to explore the association between HCV infection and HL.

3. HCV infection and breast cancer

Breast cancer is the leading cause of cancer incidence worldwide, representing 11.7% of all cancer cases, and is the fifth leading cause of cancer mortality worldwide.

It is estimated that one in eight women in the world will develop breast cancer, but the exact cause of breast cancer is still unknown [20, 21].

The studies conducted by Larrey et al. [22], Omland et al. [23], and Swart et al. [24] did not show any association between HCV infection and breast cancer. However, Su et al. conducted a case–control study in 2011, which included a total of 1958 patients with newly diagnosed breast cancer during the period 2000–2008 [25], and showed no significant difference in the prevalence of HCV infection between breast cancer patients and control subjects ($p = 0.48$). However, patients aged <50 years with HCV infection had a 2-fold greater risk of developing breast cancer, suggesting that chronic HCV infection may be associated with early-onset breast cancer. However, given the retrospective nature of this study, there may be some false-positive and false-negative linkages. Thus, prospective studies are still needed to confirm their relationship.

The mechanism of the association between HCV infection and breast cancer is unclear. It is currently believed that HCV infection damages liver tissue and thus elevates estrogen in the blood, and the liver is the only organ of estrogen inactivation and metabolism, and elevated estrogen is strongly associated with the development of breast cancer [22, 26].

4. HCV infection and cancers of the digestive system

4.1 HCV infection and pancreatic cancer

Pancreatic cancer (PAC) is one of the most aggressive and lethal cancers in humans, with a high mortality rate and an overall five-year survival rate of <5%, resulting in approximately 250,000 deaths worldwide each year. The probability of PAC increases with aging and nearly 80% of these malignancies develop in subjects aged between 60 and 80 years old. In addition, smoking and family history are also strongly associated with an increased risk of PAC [23, 24, 27].

In a study conducted by Darvishian et al., based on the British Columbia Hepatitis Testers Cohort (BC-HTC), the risk of PAC was found to be significantly higher among HCV-infected individuals, irrespective of sex [28]. However, the information on smoking status was not collected in this study, so there was some potential bias. Similarly, Arafa et al. completed a meta-analysis of the association between HCV infection and PAC in 2020 [29]. A total of 16 studies were included, including 8 case–control studies and 8 cohort studies. Eventually, 7 studies (5 cohort and 2 case–control) showed a statistically significant association between HCV infection and PAC, while 9 studies (3 cohort and 6 case–control) did not. A meta-analysis of the 16 studies revealed that HCV-positive people had 51% higher risk of developing PAC than HCV negative people. However, this association was weakened among the studies that adjusted for potential risk factors for PAC, such as diabetes, chronic pancreatitis, and alcoholism. Therefore, prospective cohort studies with more information about potential confounders are needed to confirm the conclusion.

The mechanisms of HCV infection and PAC may be as follows. Fiorino et al. proposed a comprehensive and qualitative hypothetical model, namely "tensegrity model hypothesis" [30]. HCV-mediated perturbation of this interplay causes a substantial change of critical intracellular biochemical activities as well as that of genome expression that ultimately leads to the development of cancer. Specifically, HCV proteins, including HCV NS3, NS4A, NS5A, and NS5B, can interact with cytoplasmic enzymes

and components of the cytoskeleton, leading to the disruption of cell structure, altered enzyme activity, and ultimately interference with several signaling pathways for important cell functions, such as proliferation, differentiation, energy production, and apoptosis, and qualitatively and quantitatively disrupt several intracellular biochemical activities associated with transcription, translation, and transduction of nuclear genes, leading to PAC [29, 30].

4.2 HCV infection and gastric cancer

Gastric cancer is the fifth most common cancer worldwide. Although the incidence of gastric cancer has decreased in recent years, it remains a major public health problem. Risk factors for gastric cancer include *Helicobacter pylori* infection, age, high salt intake, and low dietary intake of fruits and vegetables [31, 32].

Few studies reported the association between HCV infection and gastric cancer. Chen et al. found that HCV infection was a risk factor for gastric cancer development [33]. Since then, Yang et al. published a meta-analysis on the association between chronic hepatitis virus infection and gastric cancer in 2021, including 13 studies, five of which were on the association between HCV infection and gastric cancer [34]. The risk of gastric cancer in HCV-infected patients was increased by 88% compared with those without HCV infection ($P = 0.001$).

The mechanisms of HCV infection promoting gastric cancer can be elaborated from the following aspects. First, it has been shown that HCV acts as an indirect carcinogen of gastric cancer by promoting and maintaining a chronic inflammatory state at the site of infection, resulting in a gradual rearrangement of gastric tissue structure [5, 35]. Second, HCV inhibits antigen processing and presentation and induces a gastric mucosal oxidative stress response, which has been shown to be associated with the development of cancer. Therefore, it is reasonable to suspect that HCV infection may induce the development of gastric cancer [34, 36, 37]. Third, cirrhosis may also play an important role in the development of gastric cancer. The idea that HCV infection is a risk factor for cirrhosis has been well established, and studies have shown that cirrhosis is closely related to gastric cancer. Thus, patients with HCV infection can develop cirrhosis first, which can lead to the development of gastric cancer [38, 39].

4.3 HCV infection and cholangiocarcinoma

Cholangiocarcinoma (CCA) is a malignant neoplasm originating from biliary epithelial cells. CCA can be divided into intrahepatic cholangiocarcinoma (ICC) and extrahepatic cholangiocarcinoma (ECC), and epidemiological surveys show a gradual increase in their incidence and prevalence. Many risk factors, including primary sclerosing cholangitis, liver fluke infection, and liver stones, increase the risk of CCA [40, 41].

The earliest study on the relationship between HCV infection and CCA was conducted in Japan [42]. HCV-associated cirrhosis was a major risk factor for primary hepatobiliary cell carcinoma in Japan. Li et al. [40] and Tan et al. [43] performed meta-analyses of the relationship between HCV infection and CCA, and both of them showed that HCV infection significantly increased the risk of CCA. But a study by Zhou et al. did not find a significant correlation between them [44]. However, differences in tumor heterogeneity, misclassification, selection bias, incidence, and prevalence patterns in each country can cause bias in the meta-analysis results. Therefore, future prospective studies are needed to confirm these results.

HCV infects bile duct cells, and therefore the induction of transformation by viral oncoprotein activity may be a direct mechanism for the development and progression of CCA. Therefore, bile duct cells can express receptors and cytokines related to HCV infection susceptibility and HCV replication permissibility, leading to bile duct damage and loss, and ultimately bile duct carcinoma [45–47]. In addition, the modulation of epithelial-mesenchymal transition (EMT) and hedgehog (Hh) pathway by HCV and/or viral proteins and its resultant chronic biliary inflammation could be the indirect role of this viral infection on the process associated with fibrosis and CCA [46].

5. HCV infection and thyroid cancer

Thyroid cancer is the most common cancer of the endocrine system [48]. The incidence of thyroid cancer has steadily increased by approximately 4% per year. Important risk factors for thyroid cancer include a history of radiation exposure and a family history of thyroid cancer [49].

There are also some debates about the association between HCV and thyroid cancer. Some studies supported their association [50], while others did not [51]. To further clarify the correlation between HCV infection and thyroid cancer, Wang et al. conducted a meta-analysis in 2021, which included 6 articles, and showed that HCV infection was significantly associated with an increased risk of thyroid cancer [52]. However, only a few papers were included, and the lack of other factors, such as age and gender, may affect the incidence of thyroid cancer, thus interfering with the accuracy of statistical results.

The mechanism by which HCV promotes thyroid cancer remains unclear. However, some studies suggest that HCV may affect the immune system and the self-recognition of thyroid cells, in which HCV may directly damage thyroid tissue or mimic the structure of certain components of the thyroid gland, thereby triggering autoimmune disease. Therefore, the risk of thyroid disease may be increased after HCV infection [52, 53].

6. HCV infection and kidney cancer

Kidney cancer is the most common type of urogenital tract cancer. It has a mortality rate of 30–40% [54]. Risk factors for kidney cancer have not been identified, but high BMI, tobacco use, and hypertension influence the development of kidney cancer [55].

In recent years, there have been studies on the association between HCV infection and kidney cancer. Gonzalez et al. confirmed that HCV infection is an important risk factor for kidney cancer [56]. Wu et al. conducted a meta-analysis on the association between HCV infection and kidney cancer in 2021 [57]. The study population included 391,071 HCV patients and 38,333,839 non-HCV controls, from 9 different countries. Pooled results showed that HCV-infected patients had a significantly higher risk of developing kidney cancer. However, some well-known risk factors for kidney cancer, such as smoking, obesity, and high blood pressure, are not matched or adjusted. By contrast, in a Swedish study, no significant correlation was found between them [58]. Further clarification should be needed.

Although the mechanism by which HCV infection may lead to kidney cancer is not fully understood, HCV core protein has been found in the glomerular structures

and tubular epithelial cells of the kidney in patients with HCV infection [59]. In addition, several other hypotheses have been proposed. First, it has been found that HCV infection may be related to the NY-REN protein, an alterable ubiquitin-related protein that impairs the autophagic response through ubiquitin protein ligase-related self-regulatory mechanisms, which in turn promotes tumorigenesis [60, 61]. Second, cytotoxic T-cell-dependent apoptosis plays a pilot role in the host immunity and normal tissue. HCV can disturb this process and lead to renal oncogenesis [61, 62]. Third, serine protease inhibitor Kazal (SPIK) is a cellular protein that inhibits serine proteaserelated apoptosis in kidney cancer tissue samples as an additional mechanism for HCV induced kidney cancer [61].

7. HCV infection and other extrahepatic tumors

Very few studies have also explored the association between HCV infection and other extrahepatic tumors, such as oral cancer, skin cancer, and colorectal cancer, but their finding has not been discussed in this chapter due to the paucity of relevant data or a substantial controversy of the current conclusions.

8. Conclusion

It has been well-recognized that HCV infection is a risk factor for hepatocellular carcinoma. Due to the risk of this virus on the development of cancer, many studies have indicated an increased risk of extrahepatic tumors in HCV-infected patients. However, it should be noted that the occurrence of cancer is attributed to a combination of multiple factors, such as family genetics, race, and lifestyle. Thus, the relationship between them is still challenging. Regardless, screening for various cancers is still necessary for patients with HCV infection, except for routine prevention of cirrhosis and liver cancer.

Conflict of interest

The authors declare no conflict of interest.

Notes/thanks/other declarations

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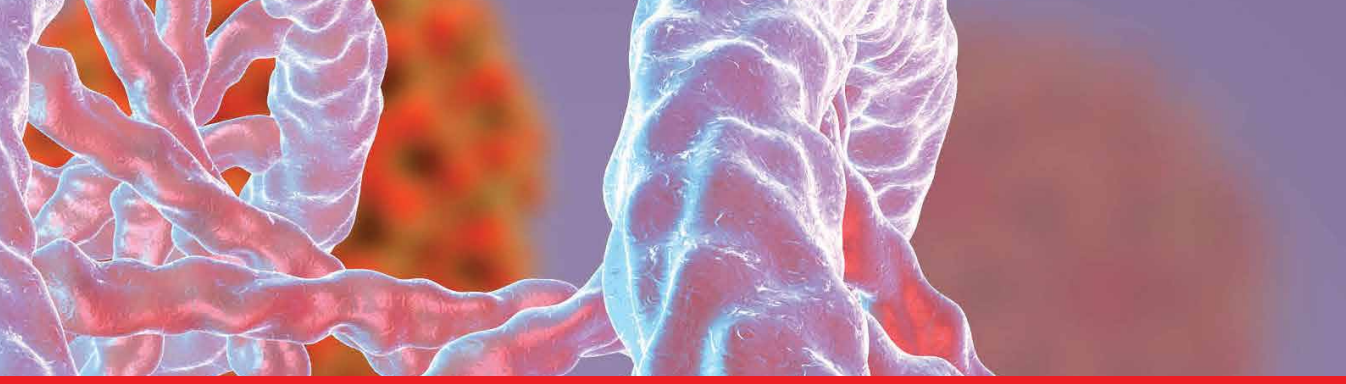

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This book provides a comprehensive overview of hepatitis C infection. It includes two sections. The first section includes four chapters that discuss the structure, diagnostic methods, phylogenetic classification, and multiscale viral dynamics modeling of the hepatitis C virus and its interaction with host genetics. The second section includes four chapters that summarize the existing strategy of hepatitis C virus elimination, management of hepatitis C virus in patients with chronic kidney disease, and association of hepatitis C virus with hepatic and extrahepatic malignancy. This book is a useful resource for investigators studying hepatitis C infection and physicians treating the disease.

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