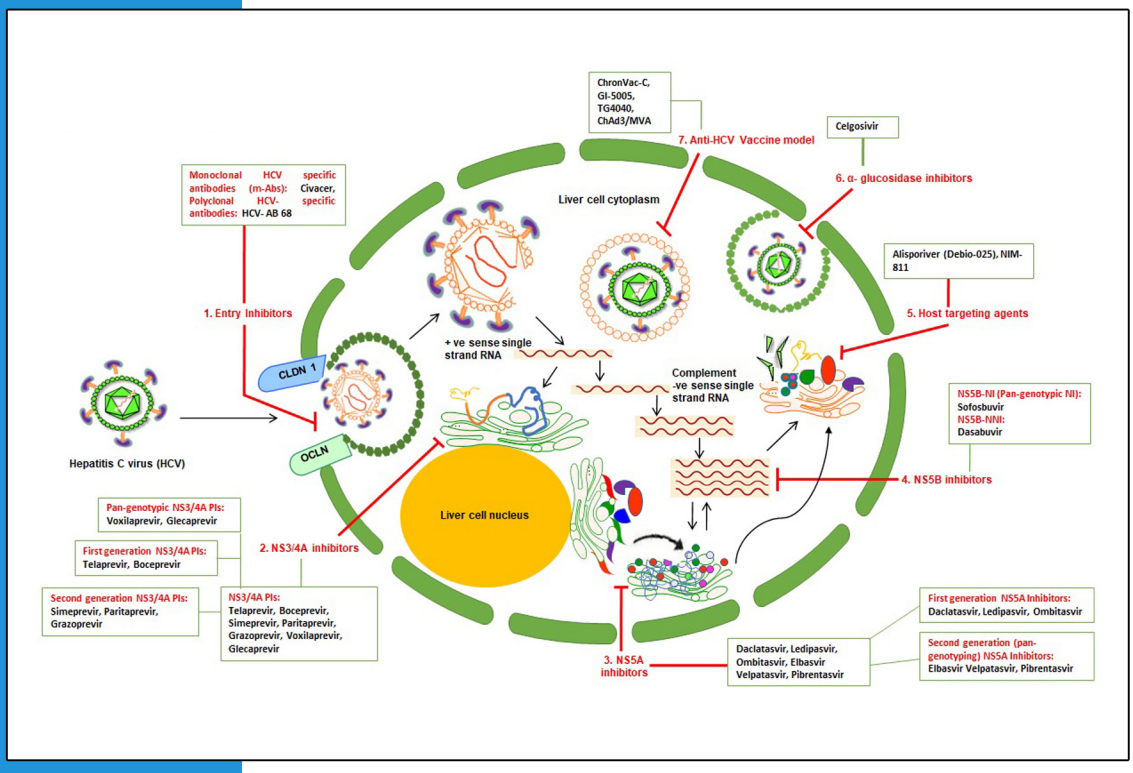
HEPATITIS C VIRUS-HOST INTERACTIONS AND THERAPEUTICS: CURRENT INSIGHTS AND FUTURE PERSPECTIVES



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Hepatitis C Virus-Host Interactions and Therapeutics: Current Insights and Future Perspectives

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FOREWORD

Virus-host interactions (dependencies) and their intricate interplay are always phenomenal and of paramount interest to investigate molecular epidemiology and pathogenesis of all viral infections. However; complex cross-talks between hepatitis C virus (HCV) and host cells involving a plethora of genes and cell signaling pathways leading asymptomatic acute hepatitis C infection to chronic hepatitis C (CHC) and further durable, sustained, and insidious extrahepatic manifestations are still mysterious, murky, inconclusive, and even more, remains to elucidate. The world has always seen paradigms in HCV infection biology and therapeutics since its discovery in the late 1980s by gene cloning methods instead of the use of conventional tissue culture, or classical virological techniques. We can say that the world could not be able to see this revolutionary advancement in molecular biology techniques, state-of-the-art diagnostic tools, sophisticated protocols of clinical trials, astute knowledge of molecular drug targets, and serendipitous design and development of drug molecules without HCV discovery that once labeled as the 'silent epidemic' afflicting even more than 58 million peoples around the globe nowadays. Before the advent and approval of all oral interferon-free direct-acting antivirals, the gold standard of care, a combination therapy including pegylated interferon plus ribavirin achieved sustained clearance of HCV and subsequent improvements in liver disease in only 60% of treated patients. It has been a long haul, a big haul, but we've made it with the current landscape of HCV therapeutics to cure harder-to-treat special HCV subpopulations including cirrhotic, decompensated cirrhotic, HCV/HIV or HCV/HBV co-infections, and liver transplant or post-transplant patients with virus recurrence. Despite oral direct-acting antivirals having started to bear fruit in real-world clinical settings against HCV, unmet challenges, barriers, and hurdles in HCV diagnostics and treatment accessibility still prevail that need to surmount and overcome to reduce the global healthcare burden of HCV and to achieve WHO striving goal of HCV elimination by 2030.

This thought-provoking book tends to cover a comprehensive and up-to-date overview of the latest advancement in HCV infection biology, perplexing molecular clinical pathology, novel diagnostic tools, fortuitous anti-HCV therapeutic options on the horizons, management of difficult-to-treat subpopulations, and imperative compensatory therapies against hepatitis C infection in the late stage of their development. The book intends to cover the latest guidelines issued by the US FDA, AASLD/IDSA (American Association for the Study of Liver Diseases/Infectious Diseases Society of America), ECDC (European Center for Disease Prevention and Control), and NYSDOH-AI (New York State Department of Health Aids Institute) on HCV screening, diagnosis, treatment and management for the clinicians, physicians, infectious disease experts, and primary care providers (nurses and paramedics). The contents of the book not only provide ABC of HCV infection, diagnostic tools, and treatment strategies to common and interested readers but also address current information about some pretty mind-boggling aspects of hepatitis C biology, immunology, pharmacology, therapeutics, and vaccinology to obtain relevant answers with thoughtful explanations of some outstanding problems in the field nowadays. The book also covers up-to-date guidelines about patient screening, treatment protocols, and optimization of therapy in real-world clinical settings.

The book also summarizes unanswered questions currently faced by investigators in the intriguing molecular pathogenesis of CHC in humans, and physicians, clinicians, hepatologists, and healthcare officials are concerned with HCV clinical diagnosis and therapeutics. I hope that this book will also be a valuable addition to hepatology for curious undergraduate and postgraduate students pursuing a new career in HCV medicine or newcomer in hepatitis C research to broaden their knowledge and gain a wide perspective of

the field. The multi- and interdisciplinary presentation of information in this book would open a new school of thought for postdoctoral fellows to search for new horizons and milestones in hepatitis C research. The book closes while shedding light on remarkable and amalgamated efforts by the World Health Organization (WHO), health policymakers, and public healthcare providers to a proper ending of this 'silent epidemic' from the world by 2030.

Both authors of the book (Imran Shahid and Qaiser Jabeen) are agile, bright, and very active investigators in the concerned field of hepatitis C research with more than 10 and 20 years of experience respectively. I wish that this web-based, as well as hard copy format book, would rapidly become an international referral and guideline in hepatitis C infection biology, diagnostics, and therapeutics.

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PREFACE

The public health care burden of hepatitis C virus infection remains significant despite the remarkable progress in HCV therapeutics during the last decade, as around 58 million people are currently having the virus and almost 4,00,000 deaths are reported annually due to hepatitis C-induced hepatocellular carcinoma. The advent and approval of a dozen oral interferon-free direct-acting antivirals have revolutionized the treatment paradigms for hepatitis C infection while achieving higher sustained virologic response rates (>95%) in treated individuals. Analogously, HCV and host interactions to map networks in disease progression, extrahepatic manifestations, and virus invasion strategies to the host immune system are still enigmatic to fully understand. Albeit, ample understanding of HCV life cycle *in vitro* and transient *in vivo* replication models, provide valuable insights towards the viral entry, genome replication, virion formation, and host infection involving a highly orchestrated series of molecular and cellular events, including a plethora of genes and cell signaling cascades. However, questions remain to answers regarding the virus clearance without any therapeutic intervention in some HCV-infected populations, molecular pathogenesis of infection transforming the acute hepatitis C infection to end-stage liver disease, post-viral eradication, hepatocellular carcinoma, and the fate of the immune system once the virus eliminates from the body.

This book intends to provide insights into what has been achieved in HCV infection biology, virus-host interactions, molecular epidemiology of the infection, and the full spectrum of immune responses to hepatitis C, either the involvement of innate or adaptive immune responses in the first three chapters. The following chapters 4 and 5 comprehensively illustrate the simplified HCV diagnostic solutions, predictive barriers, and future perspectives for general, as well as vulnerable HCV infected populations, along with the up-to-date procedural and protocol guidelines for HCV diagnosis. Book Chapter 4 also highlights the strides toward a better understanding of HCV screening and diagnostic strategies for high throughput anti-HCV screening and diagnosis, applications for risk prediction tools, and cost-effective analysis of current diagnostic in the hepatitis C cascade of care. The next four book chapters (from chapters 6 to 9) precisely and comprehensively illustrate the current landscape of HCV treatment, their administration, consensus treatment guidelines for their recommendations, and their real-world treat outcomes. These book chapters also provide the probable answers to some fundamental questions like why some HCV-infected populations are nonresponsive to IFN-free DAAs, the phenomena behind viral relapse, and reasons behind treatment failure in harder-to-treat specific HCV subpopulations. Within those book chapters, chapter 9 also emphasizes real-world challenges in HCV therapeutics to unleash certain barriers while making it possible to expand the scale of therapy, and the cascade of care, and bridge existing gaps in hepatitis C care between developed and poor nations. Chapter 10 of the book overviews the emerging anti-HCV treatment strategies in the pipeline and explains the efforts to do while refocusing on anti-mRNA-based treatment strategies and nanomedicine-based approaches used in conjunction with DAAs for hepatitis C. The book chapter also portrays some glimpses into the future for the design of controlled animal and human HCV infection models for the design of an appropriate but effective prophylactic or protective anti-hepatitis C vaccine model. The last two book chapters (chapters 11 and 12) discuss the goals, policy implementation, and progress of the Global Health Sector Strategies (GHSS) 2016-2021 on HCV worldwide in the last decade as well as the cross-cutting barriers to break and actions to be taken in this decade while achieving the global goal of HCV elimination by 2030. The final chapter of the book also describes the strategies to opt at hospitals, community services centers, health care service providers, and government levels to prevent new HCV incidences and reduce HCV-related morbidities and mortalities with

concrete efforts, collective will, and public health notes to galvanize the efforts to eradicate this epidemic worldwide by 2030.

Overall, this well-written book provides from simple, accessible introduction to a complex hepatology field, and in-depth information about HCV basics, clinical diagnostics, and therapeutic knowledge to all stakeholders involved in HCV screening, diagnosis, treatment, and management. Without being exhaustive or redundant, it can be easily accessed to concepts by extensive cross-referenced studies. We hope that this book will enhance the knowledge of a common reader about hepatitis C infection, its diagnosis, and treatment, and provide answers to the physicians, clinicians, hepatologists, infectious disease experts, health care providers, and investigators to some of the outstanding problems in the ever-changing field of hepatitis C research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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DEDICATION

Dedicated to one of my beloved teachers who told me that "To be a star in real-life, you will have to do a lot".

CHAPTER 1

HCV-Host Interactions: A Plethora of Genes and their Intricate Interplay Part 1: Virus Specific Factors

Abstract: Hepatitis C virus (HCV) interaction with host cells is pivotal for natural disease course starting from asymptomatic acute infection to progress into persistent chronic infection and subsequent extrahepatic manifestations, including fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). The HCV infection biology in infected host cells *via* virus attachment, virus genome replication, mRNA translation, new virion formation, and egress from infected cells involves highly coordinated participation of the virus- and host-specific proteins, a plethora of genes, and cell signaling cascade. The progression of persistent chronic hepatitis C (CHC) infection to hepatic fibrosis, cirrhosis, and HCC involves viral invasion strategies against host immune system defense mechanisms as well as impeding healthy metabolic and signaling networks of the liver cells. Thereby, HCV-induced liver injury *via* chronic inflammatory processes that fail to resolve is responsible for decompensated cirrhosis and on occasion, hepatocarcinogenesis in infected individuals. With the latest advancement and rapid expansion of our knowledge in hepatology, the human liver is deciphered as an immunologically distinct organ with its specialized physiological niche. The relationship between human hepatocytes and different components of the immune system is quite complex and dynamic. The immunopathogenesis of various viral infections demonstrates that the immune system plays an essential role to determine the progression of many hepatic diseases through immune cell communication and cell signaling networks. In this book chapter, we overview HCV-host interactions and their intricate interplay with complex crosstalk to propagate less fetal acute HCV infection to CHC and subsequent hepatocarcinogenesis (*i.e.* HCC) in infected individuals.

Keywords: Acute hepatitis C, Chronic hepatitis C, Cirrhosis, Cell communication, Cell signaling, Core protein, Envelope glycoproteins, Fibrosis, Genome organization, Hepatocytes, HCV life cycle, Hepatocellular carcinoma, Immunopathogenesis, Molecular pathogenesis, Nonstructural proteins, RNA polymerase, Replication, Structural proteins, Translation, Virus-host interaction.

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INTRODUCTION

Hepatitis C Virus Taxonomy

HCV belongs to the *Flaviviridae* family of viruses which is divided into three genera: flavivirus, pestivirus, and hepacivirus [1].

Flaviviruses include yellow fever virus, dengue fever virus (DENV), Japanese encephalitis virus, and Tick-borne encephalitis virus [2]. Pestiviruses include the bovine viral diarrhea virus, classical swine fever virus, and Border disease virus [1]. HCV, with 8 genotypes (GTs) and numerous subtypes (around 70), is a member of the *hepacivirus* genus, which includes tamarin virus and GB virus B (GBV-B) and is closely related to human virus GB virus C (GBV-C) [3]. All currently identified HCV GTs and subtypes are hepatotropic, which means that they preferentially infect hepatocytes and cause liver inflammation, broadly known as viral hepatitis C infection [3]. However, viral infectivity and pathogenicity do vary at GTs and subtypes level, which may variably influence the progression of HCV infection from the acute phase to chronicity and subsequent hepatic-comorbidities in infected individuals [4]. Interestingly, all members of the *Flaviviridae* family of viruses shares and resembles several basic structural and virological characteristics [1]. At the genome level, all *Flaviviridae* viruses are single-strand RNA viruses from 9600 to 12,300 nucleotides (nts) in length, with an open reading frame (ORF) encoding a polyprotein of 3000 amino acids (aa) or more [5]. The viral genome is flanked by a 5' untranslated region (5' UTR) upstream to the structural part of the ORF of 95-555 nts and a 3' untranslated region (3' UTR) of 114-624 nts in length adjacent to nonstructural proteins [5]. Structurally, the HCV virus is enveloped in a lipid bilayer in which two or more envelope glycoproteins (*i.e.* E1 and E2) are anchored [6]. The virus envelope surrounds the nucleocapsid, which is composed of viral antigen protein (*i.e.* core or C; multiple copies of a small basic protein) and the viral RNA genome [6]. The viral structural proteins (*e.g.*, C, E1, and E2) are encoded by the N-terminal part of the ORF, whereas the 7 nonstructural proteins (*e.g.* P_7, NS1, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) are constituted by the remaining portion of the ORF [6]. Surprisingly, motifs-conserved across RNA protease helicase (*i.e.* nonstructural protein; NS3) and RNA replication enzyme (*i.e.* nonstructural protein NS5B; an RNA-dependent RNA polymerase (RdRp)) are found in similar locations in the polyproteins of all of the *Flaviviridae* viruses [6]. In addition to that, the hydropathic profile of polyproteins is much closer/identical between flaviviruses and hepaciviruses than pestiviruses [1].

Likewise, with the above-mentioned similarities, HCV exhibits several virological, epidemiological, and pathophysiological differences from the other

members of the *Flaviviridae* genera [1]. Flavivirus translation is cap-dependent, *i.e.* it is mediated by a type 1 cap structure located in the 5' UTR (m^7GpppAmp), followed by the conserved AG sequence and a relatively short stretch upstream of the polyprotein coding region [7]. In contrast, HCV translation is cap-independent, where the 5' UTR is uncapped and folds into a complex RNA secondary structure adjacent to a portion of the core-coding domain [7]. Almost all 5' UTR and a few upstream nucleotides of the core-coding domain are occupied by several stem-loops (SLs) of internal ribosome entry sites (IRES) which span a region of ~341 nts and mediate direct binding of ribosomal subunits and cellular factors and initiate subsequent translation [6]. 3' UTR of the other members of flavivirus is highly structured, however; HCV 3' UTR is relatively short, less structured, and contains a poly-uridyl tract that varies in length and plays a central role in HCV replication [6].

HCV: Route of Transmission

The major route of HCV transmission is exclusively through direct blood-to-blood contact between humans which signifies its narrow tissue tropism and host specificity for HCV infection [8]. However, some other body fluids and tissue specimens are also capable of transmitting HCV infection (Table **1**). Flaviviruses principally infect a broad range of vertebrate animals by using mosquitoes or ticks as a virus vector, and humans are dead-end host that does not participate in the perpetuation of virus transmission [9]. Pestivirus can rarely infect humans and no known insect vector has been identified until now [10]. Most of the infections caused by flaviviruses are acute-limited in vertebrate animals whereas HCV in humans has a high chronicity rate (50%-80%) depending on the age at infection [11]. Protection and recovery of flavivirus and pestivirus infections are entirely dependent on strong and adapted humoral and cellular immune responses [10]. However; in CHC and subsequent extrahepatic manifestations of HCV infection (*i.e.* cirrhosis, HCC), virus-induced host-immune responses fail to confer protection and prevention to reinfection with homologous and heterologous strains of HCV in the infected individuals [11].

HCV Genome Organization

HCV is a positive-sense single-stranded RNA virus of approximately 9600 nucleotides in length [7]. The viral genome flanks by highly conserved 5' and 3' UTRs along with a single ORF of 3010 to 3037 amino acids polyprotein [7]. Both 5' and 3' UTRs are pre-requisite for virus replication and mRNA translation and signals required for both these phenomena are coordinated in a highly orchestrated manner within viral and cellular proteins and between these two regions of the HCV genome (*e.g.*, molecular interaction of IRES domain IIId,

CHAPTER 2

HCV-Host Interactions: Interplay Part 2: Host Related Determinants and Intracellular Signaling

Abstract: The progression of acute HCV infection to chronic disease and subsequent extrahepatic comorbidities involve both viruses and host cellular proteins interactions as well as insurrection or subjection of cell signaling and metabolic pathways in infected cells. This interaction between host-specific factors and the hepatitis C genome also weakens or impairs other physiological or metabolic regulatory roles of the hepatocytes. Several host cell proteins promote hepatitis C infection through binding to HCV nonstructural proteins (*e.g.*, PPP2R5D). Some studies also found cytokine (*e.g.*, IL-10, IL-6, TNF-α, and TGF-β1) gene polymorphisms to be highly associated with chronic hepatitis C (CHC) infection progression, whereas, polymorphism in some host genes (*e.g.*, PNPLA3, ADAR-1, and IFIH1) are found to be actively involved in the induction of advanced liver fibrosis in patients co-infected with HIV-1/HCV. Host lipid metabolism reprogramming through host lipid regulators (*e.g.*, ANGPTL-3 and 4) is also considered essential for CHC progression to severe liver disease (*e.g.*, cirrhosis and HCC). Several microRNAs (*e.g.*, miR-122, miR135a) are supposed to be key mediators of HCV infection progression and development of HCC in infected individuals and associated hepatic comorbidities. In chapter 1, we have illustrated the potential roles of virus-specific proteins in HCV molecular pathogenesis. Herein, we will elucidate the host-specific culprits that subvert, impede or disrupt host cells' communications, cell signaling, and metabolic pathways to propagate HCV infection. We will also elaborate that how the subversion of infected host-cell signaling and metabolic pathways disrupt cellular networks to evolve advanced fibrosis and hepatocarcinogenesis in HCV-infected individuals.

Keywords: ANGPTL-3, ANGPTL-4, Cytokines, Cell signaling, FAS ligand, Gene polymorphism, Hepatocellular carcinoma, Host factors, Interferon lambda 4, Lipid metabolism, Liver cancer risk, Liver fibrosis, MicroRNA, MiR-122, MiR-135a, Metabolic pathways, Nuclear factor-ƙB, PPP2R5D, Toll-like receptors, Tumor necrosis factor-α.

INTRODUCTION

Hepatitis C infection causes the inflammation of the liver and subsequently progresses hepatic injury into severe comorbidities like fibrosis, cirrhosis, and hepatocarcinogenesis [1]. Chronic HCV infection is associated with rapid disrup-

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tion of normal physiological and regulatory roles of hepatocytes while eliciting or suppressing the expression of certain host factors, by modulating cell signaling and impeding metabolic networks [2]. Different HCV genome regions interact with hepatic cellular factors, cell signaling molecules, and metabolic pathways to weaken or impair the normal function of the liver in infected individuals [2]. Many host-specific factors have been identified to be involved in and regulate HCV RNA replication by different mechanisms including the disruption of viral proteins, RNA, or the viral replication complex. Indeed, the hepatitis C infection progression in hepatocytes displays a landscape of complex virus-host interactions, and unleashing those host factors is very critical to fully elucidate the mechanism of HCV RNA replication and viral pathogenesis [3]. Furthermore, genetic polymorphisms of pro- and anti-inflammatory mediators can also modulate the susceptibility of hepatitis C infection in certain populations [4]. In addition to that, differential expression of some host lipid regulators in HCV infection may contribute to post-treatment deregulation of lipid metabolism that could predispose certain individuals to HCC development [5]. In this book chapter, we discuss how host cellular factors deregulate, disrupt, or impede normal hepatic cells mechanisms upon interaction with different viral proteins which subsequently are associated with or correlate to propagate hepatitis C infection into severe hepatic diseases like hepatic fibrosis, cirrhosis, and HCC.

CERTAIN HOST SPECIFIC FACTORS ARE VITAL FOR HCV REPLICATION

PPP2R5D (Protein Phosphatase 2 Regulatory Subunit B'Delta) Protein

NS5B protein is essential for HCV replication either *in vitro* or *in vivo* and certain host factors have been reported to interact with NS5B to augment virus replication or production by different mechanisms [6]. Multiple studies report the identification and demonstration of many cell culture adaptive mutations in NS5B protein to initiate or enhance the viral RNA replication or virus production during the development of infectious HCV recombinants and full-length clones. These mutations were also proposed to confer resistance or reduce the efficacy of HCV antivirals in bioinformatics studies. Three key mutations, F1464L/A1672S/ D2979G (LSG) in NS5B protein, permitted the replication of full-length HCV clones of GT-2a and 2b isolates, and the infectious clones of GT-1a, 2c, 3a, and 6a are reported in the literature [7]. However, the full mechanistic role of LSG mutations to accelerate HCV replication is still elusive [7]. Recent studies predict that HCV NS5B interacts with cellular PPP2R5D (protein phosphatase 2 regulatory subunit B'Delta) protein while initiating HCV replication and infection. PPP2R5D is a subunit of PP2A (protein phosphatase 2A) enzymes, which are involved in many regulating pathways and turn on or off gene expression by

removing the phosphate group from proteins [8]. PP2A is a major and multifunctional serine/threonine-specific phosphatase consisting of a structural subunit A, a regulatory subunit B, and a catalytic subunit C [8]. PPP2R5D plays a major role in negative regulation of the PI3K/AKT signaling pathway, autism, or other brain-related disorders [9]. PP2A has been evaluated in cancer progression and this group of proteins has shown interaction with different HCV proteins [10, 11].

Micro RNAs (miRNA)

Conventionally, miRNAs (small non-coding RNAs of approximately 22 nucleotides in length) are considered important regulators of gene expression by suppressing mRNA translation and promoting mRNA decay [11]. However, contrary to their conventional suppressive role, certain liver-specific miRNAs anneal to HCV genome replication machinery and play an essential role in viral infection progression. A liver-specific microRNA (*i.e.* miR-122; which accounts for 70% of all hepatocytes miRNAs), affects both the initiation and maintenance of HCV infection [12]. Regulation of HCV liver tropism is almost dependent on miR-122 because of its liver-specific expression and is the most abundant miRNA in the hepatic cells [12]. It anneals to the HCV genomic 5' UTR and is vital for virus replication; albeit, the mechanism by which miR-122 promotes virus replication and genome amplification is not well understood and is still debated [12]. However, recent studies show that miR-122 increases the rate of viral RNA accumulation and promotes the establishment of hepatitis C infection in a greater number of hepatic cells than in the absence of miR-122 [12]. For this purpose, it associates with human Argonaute proteins (Ago 1-4; hAgo:miR-122 complex), which further binds to two sites on the upstream nucleotides of 5' UTR of the viral genome and promotes viral RNA accumulation [12]. Some other roles of miR-122 have also been identified, including viral translation stimulation, genome stabilization, and a direct role in genome amplification. Recent studies suggest that miR-122 is involved in the formation of an active or a translation-favorable viral IRES structure by modulating the 5' UTR shapes during its annealing with 5' UTR nucleotides; however, biophysical analyses are extensively required to support this hypothesis [12]. Furthermore, wild-type HCV genome replication cannot be detected in the absence of miR-122. However, certain studies document extrahepatic HCV RNA existence and replication in other tissues of the body where CHC propagates to a broad range of comorbidities including cryoglobulinemic vasculitis, diabetes Mellitus, and B-cell lymphoma. It suggests a possible role of miR-122-independent HCV replication and virus accumulation in these clinical manifestations [12].

CHAPTER 3

Immune Responses and Immunopathology of Acute and Chronic Hepatitis C Virus Infection

Abstract: An ample understanding of the HCV life cycle and infection biology has also significantly increased our knowledge of hepatitis C immune responses against acute infection to the progression of chronic hepatitis C and associated comorbidities. As expected in chimpanzees (the best *in vivo* model so far to study hepatitis C infection kinetics, molecular pathogenesis, and immunopathology) and humans, several arms of the immune responses are activated following HCV infection. Some of the underlying mechanisms both for innate immune responses and adaptive immune responses to viral clearance and persistent HCV infection are fully understood, however; some fundamental questions in hepatitis C immunopathology remain to be answered and some immune responses hypothesis demands further studies to validate. Some mechanistic issues of viral evasion strategies during infection progression and the future development of prophylactic and protective anti-HCV vaccines will be largely dependent on the full understanding of the kinetics of adaptive immune responses against HCV infection. As generally presumed the inefficient role of innate immunity in self-resolving HCV infection, the potent immune responses of CD8+ T and CD4+ T cells are critically important after the acute phase of the infection. In particular, the plausible understanding of CD4+ T cells responses against persistent infection will certainly be central to the development of future HCV vaccines. In this chapter, we overview the host immune responses against hepatitis C acute infection and subsequent CHC infection, their regulation by viral and cellular proteins, and the virus purging strategies while impairing host defense system mechanisms.

Keywords: Adaptive immune response, Anergy, Anti-HCV vaccines, Cross-reactive neutralizing antibodies, CD+ 4 T cells, CD+ 8 T cells, Dendritic cells, Cytotoxic T lymphocyte, Hepatitis C virus, Host-immune response, Immunoregulatory cells, Immunopathogenesis, Innate immune response, Natural killer cells, Priming, Protective vaccines, Prophylactic vaccines, T-cell exhaustion, Viral escape.

INTRODUCTION

Currently, all oral IFN-free DAAs are the treatment of choice for HCV infection, where the proportion of cured patients with high SVR rates has markedly increased in real-world clinical settings [1]. However, therapy costs, treatment

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access to poor nations, chances of re-infection after post-completion therapy, and the occurrence of some severe adverse events (SAEs) are big challenges to surmount the public healthcare burden of HCV [2]. There will be always a need for prophylactic or protective HCV vaccines to control the further dissemination of infection in populations and to reduce new HCV incidences. For this purpose, extensive knowledge and a comprehensive understanding of anti-HCV immune responses against acute and chronic hepatitis C infection, viral clearance, and viral reinfection would be required and pragmatic research approaches must be implemented in various disciplines of HCV immunopathology [3]. In addition to that, the current literature is unlikely and largely decipher the HCV immune responses in disease progression phenomena rather than in host protection [3]. Second, HCV immunology research is hampered by several difficulties. First, as mentioned in Chapter 1, acute hepatitis C infections are often asymptomatic, and therefore difficult to follow the evolution of the disease before it becomes chronic. Second, most HCV immunopathological studies and their conclusions have been based on the analysis of hepatitis C immune responses in peripheral blood, which might not be representative of the anti-HCV immune responses in the liver and secondary lymphoid organs. Finally, the lack of an efficient animal model for HCV infection studies also hampers the efforts to fully understand the host's innate and adaptive immune responses against the virus. In this chapter, we briefly overview the host's innate and adaptive immune responses against HCV and its impact on preventing disease severity and chronicity. We will also elaborate on HCV evasion strategies against host immune responses during the progression of acute hepatitis C to CHC infection. In the end, we will demonstrate the importance of anti-HCV immune responses in the development of a prophylactic or protective hep C vaccine.

THE TYPICAL TIMELINE OF HEPATITIS C VIRUS INFECTION

HCV is a blood-borne virus, so the major infection route is via the blood. Some patients who are exposed to the virus develop overt liver disease, but most of them remain in a latent state [4]. Within six months of being infected, 30% of the patients expel the virus naturally while the remaining 70% enter a phase of persistent infection [4]. Once patients enter this later phase, it is very rare for the virus to be expelled naturally, with the estimated annual rate being less than 0.2% at most. Many of the patients with persistent infection show the medical conditions of CHC and develop cirrhosis in 20 to 30 years [5]. Patients with cirrhosis develop HCC at a very high annual rate of 8%, while patients with early CHC do so at an annual rate of only 0.5% (Fig. **1**) [5]. In the absence of high levels of immunosuppression, HCV is a non-cytopathic virus. In the absence of an ongoing immune response, it infects and persists in target cells, predominantly hepatocytes, without inducing inflammation and damage [3]. This has been

demonstrated in both experimental chimpanzee models and drug users or humans who were accidentally infected with contaminated needles during healthcare practice [3]. In these studies, increases in viremia were not associated with parallel increases in serum transaminase levels, thus reflecting an absence of hepatocyte damage [6]. Furthermore, following liver transplantation, the infection does not lead to liver damage for 3 weeks despite high levels of the virus [7]. The only cytopathic lesion that has been directly ascribed to HCV is the association of GT-3 infection with steatosis, the accumulation of lipids in hepatocytes [8]. Although non-cytopathic, gene microarray analysis of infected chimpanzee liver has revealed that the virus induces the expression of a large number of cellular genes as soon as a few days after HCV infection [3]. These genes include those involved in lipid metabolism and several genes associated with innate and adaptive immune responses [3].

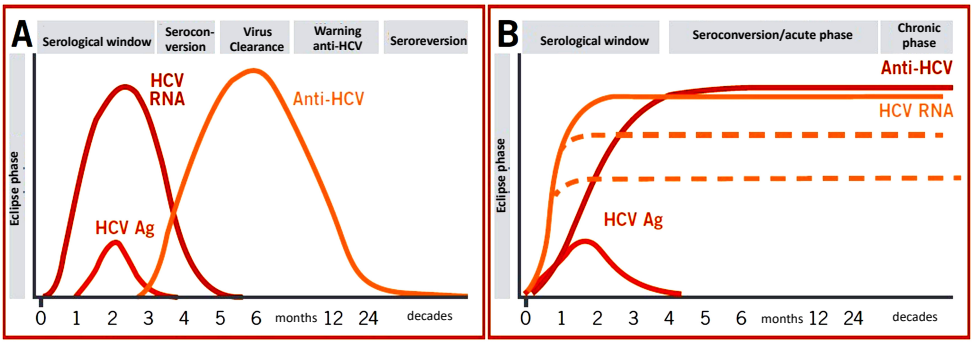

Fig. (1). Approximate time course of virological and immunological markers of hepatitis C infection [35]. **(A)** deciphers the modulation of virological and immunological markers during acute HCV infection whereas **(B)** explains the persistence of immunological markers during chronic infection. Ag; antigen.

INNATE AND ADAPTIVE IMMUNE RESPONSES AGAINST HEPATITIS C

Both the innate and adaptive immune systems appear to play an important role in the immune response to HCV [3]. The biological defense mechanism of higher organisms, including humans, is generally divided into innate immunity and adaptive immunity [3]. In the adaptive immune response, gene rearrangement by T cells and B cells enables the establishment of a defense mechanism of high specification against "the molecular microstructure of a foreign substance," and this mechanism is immunologically memorized [3]. However, as it takes a few days to induce adaptive immune responses, innate immunity works as an early defense mechanism [3]. Cells involved in innate immunity include macrophages, neutrophils, natural killer (NK), natural killer T (NKT) cells, and gamma delta (γδT) cells. Important humoral factors include complements, lectins, and

CHAPTER 4

Consensus-based Approaches for Hepatitis C Screening and Diagnosis in General and Vulnerable Populations

Abstract: Hepatitis C screening and diagnosis are both pre-requisite to predicting infection endemicity, transmission risks and identifying vulnerable hepatitis C infected populations in highly endemic areas of the infection prevalence. It is also pivotal to select optimal treatment choices and their impact, including cost and access to care, especially in resource-constrained areas in an era of all oral interferon-free direct-acting antivirals. Furthermore, hepatitis C screening is also very crucial to "find the missing millions" to achieve the hepatitis C elimination goal by 2030. It seems only possible by implementing new screening and diagnostic approaches like RNA point-of-care (RNA-POC) testing, rapid diagnostic tests (RDTs), and dried blood spot (DBS) sample testing, especially in remote communities having poor health infrastructure and where phlebotomies are a major concern for samples collection from patients who inject drugs (PWIDs). In addition to that, it is also very much required to bring HCV diagnostic facilities to decentralized healthcare centers which provide care for people at high risk or opportunistic infection of hepatitis C transmission by sexual contacts (*e.g.,* men who have sex with men (MSM), sex workers, current or former IDUs, people who are incarcerated, and people in drug harm reduction centers). In this book chapter, we will discuss consensus-based recommendations and approaches for hepatitis C screening and diagnosis in general and vulnerable populations with their potential significance for the identification and diagnosis of high-risk individuals of hepatitis C transmission. We will also emphasize the importance of initial HCV screening before the start of HCV treatment.

Keywords: Baby Boomers, Centralized HCV Testing, Disease Assessment, Decentralized HCV Testing, Dried Blood Spot, ELISA, Fibroscan, HCV Core Antigen Detection, Hepatitis C Virus, Hepatitis C Screening, High-risk Individuals, MSM, Nanoparticle-based Diagnostics, Patients who Inject Drugs, Point of Care Testing, Qualitative RNA, Quantitative RNA, Rapid Diagnostic Tests,task-shifting, Vulnerable Populations.

INTRODUCTION

Since the hepatitis C virus discovery in 1989, remarkable progress has been made in hepatitis C diagnosis from the traditional methods of anti-HCV antibodies det-

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ection (anti-HCV Abs) in plasma samples by enzyme-linked immunosorbent assay (ELISA) to the emerging state-of-the-art diagnostic tests including RNA-POCT and novel diagnostic strategies like rapid RDTs and DBS sampling used in both clinical and community settings [1]. Furthermore, qualitative and quantitative RNA viral load assessment before therapy, during therapy, and post-treatment completion by some sophisticated polymerase chain reaction (PCR) machines and real-time PCR analysis are also valuable, reliable, and authentic predictors of DAA choice for treatment initiation, to select dosage algorithms, and treatment outcomes in infected individuals [1]. These state-of-the-art screening methods and diagnostic procedures are also helpful to identify the recurrence of viral breakthroughs, reinfections, and viral relapse in treatment-failure patients [2]. In addition to that, hepatitis C RNA sequencing by traditional methods like Sanger sequencing, as well as innovative current ones by next-generation sequencing (NGS), are also very pragmatic to detect the emergence of resistance-associated substitutions (RAS) in treated patients or treatment failure patients with IFN plus ribavirin (RBV), and treatment-experienced patients without any virologic responses or virologic failure with IFN-free DAAs [1]. Besides that, hepatitis C screening and diagnosis are very useful to find many hepatitis C-infected anonymous individuals in hard-hit communities as well as in vulnerable populations who do not know their infection status and cannot afford high-cost screening in governmental and private clinical settings [1]. In this chapter, we overview the current status of hepatitis C screening and diagnostic algorithms in general and vulnerable infected populations, why we need large-scale hepatitis C screening in highly endemic areas, recommendations, and strategies to screen everyone for hepatitis C to prevent further transmission, and to decide treatment choices for those who need on prioritizing basis.

GRADING OF EVIDENCE AND RECOMMENDATION

International health organizations, policy-making committees, and research organizations provide evidence-based clinical recommendations for the physicians, clinicians, health care providers (HCPs), infectious disease experts, and primary care providers (PCPs) who refer to screening, diagnosis, and treat patients with acute and CHC infection (Table **1**). These recommendations are entirely based on comprehensive medical literature reviews and demonstrate consensus among panels of HCV experts. Each recommendation is weighed out for the strength and quality of the evidence (Table **1**) [3]. However; for the recommendations based on an expert opinion, the rationale for the opinion is included.

Table 1. Grading of evidence and recommendations for HCV screening and diagnostics [4].

Strength of Recommendation	Quality of Supporting Evidence
A = Strong	1 = ≥ 1 randomized trial with clinical outcomes and/or validated laboratory endpoints.
B = Moderate	2 = < 1 or more well-designed, nonrandomized trial or observational cohort studies with long-term clinical outcomes.
C = Optional	3 = Expert opinion

RECOMMENDATIONS BEFORE SCREENING AND DIAGNOSIS OF HEPATITIS C VIRUS

The core principles prior to screening and diagnosing an HCV-infected individual are based on who to test, where to test, and how to test. By in large, worldwide, HCV screening and diagnosis are provided in centralized lab settings, decentralized testing premises, POCT, and integrated testing approaches/ facilitation (Fig. **1**) [5].

Fig. (1). Key components of integrated testing of HBV, HCV, and HIV [5].

CHAPTER 5

The Current Paradigms of Hepatitis C Diagnosis and Innovations in the Pipeline

Abstract: Considering advances in hepatitis C therapy, global management of HCV infection becomes practicable, but some influential factors, like the capacity of countries to identify and proper diagnosis of infected individuals with immense HCV genotypic variations among different global regions and at-risk populations, cannot be passed over. Approximately, 71 million people are infected with chronic HCV infection and about 80% of them remain undiagnosed. Standard protocol for HCV diagnosis includes a preliminary serological (HCV antibody) test accompanied by an expensive confirmatory test for HCV RNA detection in serum samples of patients. However, gaps remain in the accessibility, affordability, and availability of gold-standard HCV diagnostic strategies. In pursuance of achieving the goals of the World Health Organization (WHO) for HCV elimination as a public health threat by 2030, efficient, reliable, and simplified diagnostic pathways are needed to unveil. As such, simplified sensitive strategies that can enhance the single-test diagnostic approach might assist linkage to care and direct-acting antivirals (DAAs) treatment uptake. Herein, we will discuss a few advanced diagnostic approaches to subdue some of these constraints. HCV self-testing and digital devices for the detection of HCV infection would be of prime importance in the near future. Furthermore, the availability of smart, robust, and mobile diagnostic platforms to find the missing millions in harder-to-reach populations and vulnerable individuals would also be required to link every diagnosed one with cascades of care. We will briefly cover all aspects of HCV screening and diagnostic algorithms in this book chapter along with potential advantages and disadvantages.

Keywords: Core Antigen Assay, Dried Blood Spot, HCV RNA Detection, HCV EIA Assays, Multi-disease Analyzers, Nanocomposites, Point-of-care Testing, Quick portable Tests, Rapid diagnostic Tests, Real-world Challenges, RNA POC Accuracy, Scale-up HCV Screening.

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INTRODUCTION

HCV Serology and RNA Detection

The gold standard diagnostic pathway for screening and detection of active HCV infection is based on three techniques that might be used alone or in combination [1]. These techniques include; 1) IgG antibody detection by ELISA 2) HCVcAg detection 3) and HCV RNA detection *via* PCR. HCVcAg and HCV RNA detection techniques are used to confirm viral active infection (Table **1**) [1]. HCV antibody detection approach includes the false-positive results and the diagnosis of individuals who have cleared the active infection either automatically or through antiviral treatment. Therefore, HCVcAg and viral RNA detection techniques are used for the confirmation of active infection [2, 3]. Though the specificity and sensitivity of HCVcAg testing depict remarkable performance when compared with PCR assays; however, there are still some uncertainties over whether the antigen detection test is adequately accurate to be broadly executed as a central diagnostic tool [2 - 4]. Therefore, recent WHO guidance rules recommend the utilization of HCV antibody tests as a preliminary screening process [5]. A cautious balance between cost management and specificity optimization with high sensitivity must be made [6, 7]. In a study performed by Adland *et al.*, gold standard algorithm techniques were compared among infected patients. HCV antibody testing got 87% PPV when compared to repeat antibody testing in a reference laboratory. Thereby emphasizing the increased potency for false-positive results. HCVcAg testing depicted 100% PPV in correlation with HCV RNA detection assay in multiple recent studies. Similarly, a significant correlation ($P < 0.0001$) was found between quantitative HCVcAg and viral RNA load [8]. Briefly, HCVcAg testing performs well as a diagnostic tool in comparison to RNA PCR for the identification of HCV replication.

Numerous diagnostic techniques are being evaluated regarding their multiple parameters to develop potent, rapid, and simplified HCV testing techniques. Being not exhaustive and avoiding iteration, we are discussing here novel HCV diagnostic platforms with their sensitivity, specificity, and efficacy in real-world clinical practices as well as their potential advantages and disadvantages (Table **2**). However, advanced research is further required in the field of multiple testing techniques, diagnostic platforms, and assay validation to accurately test for different simples, the diagnostic platforms which are robust, and assays that could be pragmatic in real-life HCV diagnostics.

Table 1. A summary of HCV serological and confirmatory tests with their interpretation for acute or recent infection, confirmed but unspecified resolved or chronic infection, and confirmed infant infection [10, 44].

Confirmed Case: Acute or Recent Infection	Detection of hepatitis C virus antibodies (anti-HCV) or hepatitis C virus RNA (HCV RNA) in a person with discrete onset of any symptom or sign of acute viral hepatitis within 6 months preceding the first positive HCV test **AND** • negative anti-HAV IgM, and negative anti-HBc IgM or HBsAg tests **AND** • serum alanine aminotransferase (ALT) greater than 2.5 times the upper normal limit **OR** • detection of hepatitis C virus antibodies (anti-HCV) in a person with a documented anti-HCV negative test within the preceding 12 months **OR** • detection of hepatitis C virus RNA (HCV RNA) in a person with a documented HCV RNA negative test within the preceding 12 months.
Confirmed Case: Unspecified (including chronic and resolved infections)	Detection of hepatitis C virus antibodies (anti-HCV Abs) **OR** detection of hepatitis C virus RNA (HCV RNA).
Confirmed Case: Infants < 18 months**	PCR positive for HCV-RNA^.
HCV PCR is important as viremic individuals will be considered for antiviral treatment and is a useful diagnostic tool in immuno-compromised individuals who might not mount an antibody response.	
**In infants < 18 months of age, anti-HCV testing should not be performed as the presence of anti-HCV may represent a passive maternal antibody. The cord blood should not be used because of potential cross-contamination with maternal antibodies. ^ If testing for HCV-RNA is done, it should be delayed beyond 4-12 weeks in order to avoid false-negative HCV-RNA test results.	

Table 2. The WHO essential diagnostic list of current and emerging HCV diagnostic platforms and tests with WHO prequalification, CE marked, and those which are in late-stage development [10, 42]. ND = not defined, PQ = prequalification, CE = Conformitè Europëenne, LMICs = low-to-middle-income countries, CE-IVD = Conformitè Europëenne in vitro diagnostics, POC = point of care, NAT = nucleic acid testing, HAV = hepatitis A virus, IVD= in vitro diagnostics, TB = tuberculosis, GTs = genotyping, TK = to come.

HCV Enzyme Immunoassay (HCV EIA)							
Test Name	**Sample Type/Setting**	**Turnaround Time**	**Multiplexing**	**Price**	**Regulatory Status**	**WHO PQ Review**	**Suitability**
INNOTEST HCV Ab IV	Serum, plasma	179 min	Yes (HIV & other markers)	ND	WHO PQ	-	Central labs
INNO-LIA HCV Score	Serum, plasma	1 day	No	ND	WHO PQ CE-marked	-	Strip-based method but requires cold chain and other small equipment

CHAPTER 6

Current Landscape of HCV Therapeutics

Abstract: During the last decade, the advent and approval of almost a dozen all-oral interferon-free direct-acting antivirals (IFN-free DAAs) to cure hepatitis C-infected general and harder-to-treat populations have entirely changed the treatment paradigms against this "silent epidemic". The clinical trials of generic IFN-free DAAs, while achieving 95% to 100% sustained virologic response rates (SVRs) in treated individuals, have proven their worth as "magic pills" in hepatitis C therapeutics. Following their real-world clinical usage data with SVR rates, more than 95% have raised the hopes to treat everyone infected with hepatitis C in near future, albeit certain barriers still need to be broken. These regimens, in combination or as a fixed-dose combination (FDC) of a single pill, are highly efficacious against all major hepatitis C genotypes and sub-genotypes. Furthermore, the regimens are well tolerable, with fewer adverse events, and with lesser chances of post-treatment viral relapse or breakthrough in treated patients. The dose algorithms are well-defined for all adult patient groups and in different pathological states of the infection and their recommendations are according to extrahepatic manifestations of hepatitis C in infected individuals. Furthermore, the clinical trials of some DAAs are underway to approve their recommendations in HCV-infected infants, children, and pregnant female patients. In this chapter, we will illustrate the most attractive pharmaco-characteristics of these novel therapeutic regimens to be considered while treating hepatitis C-infected populations. We will also elaborate on the infected subpopulations for which such regimens are not recommended and further research is extensively needed.

Keywords: Cirrhosis, Direct-acting antivirals, Fibrosis, Hepatocellular carcinoma, HCV/HBV Co-infection, HCV/HIV Co-infection, HCV/CKD Co-infection, Harder-to-treat populations, HCV Therapeutics, Resistance-associated substitutions, Sustained virologic response, Treatment-naïve patients, Treatment-experienced patients, Treatment-associated adverse events.

INTRODUCTION

The overall cure rates to once 'gold standard of care' (*i.e.*, pegylated interferon (PEG-IFN α) and ribavirin (RBV)) have been dismally poor with dual therapy completion in chronically infected HCV patients along with the emergence of severe adverse events [1]. All oral interferon-free direct-acting antivirals (DAAs) were awaited eagerly not only to avoid the administration of PEG-IFN α to intole-

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rant interferon patients but also to decrease the frequency of associated adverse events and viral breakthroughs (HCV RNA remains the lower limit of quantification, but increased to ≥100 IU/ml or ≥1 $\log_{10}$ during PEG-IFNα/RBV dual therapy) in infected patients [2]. The current therapies are not only safe and highly effective, but the relative ease of administration also allows for widespread use outside of a specialist's office. These oral IFN-free antivirals are reported to achieve sustained virologic response rates (SVR; undetectable hepatitis C viral load at week 12 or 24 at the end of therapy) higher than 95% in treated individuals [3]. With the availability of these safe and highly effective (yet costly) drugs, the clinical question becomes "treat selectively or treat everyone?" Furthermore, the therapeutic regimens are well tolerated, express unrestricted genotype efficacy, and result in excellent on-treatment virologic responses [3]. However, the successful use of these therapeutic regimens would depend on various factors including; previous patient history of treatment, HCV viral load monitoring during treatment, additional adverse events, possible drug-drug interactions (DDIs), and the emergence of viral escape mutants [3]. These treatment strategies are being investigated for HCV by deriving some established findings in the treatment of HIV infection, where the combination of different drugs with different anti-viral efficacy and antiviral resistance substantially decreases the risk of viral breakthroughs [4].

Although the current status of anti-HCV drug development and therapeutics is a dense, ever-changing menu of new agents, it is beyond the scope of this book chapter to cover all relevant studies. Even then, the bottom-line message from this matter-of-fact book chapter is simple: "Agent X (fill in the blank) leads to the achievement of high SVRs and cure hepatitis C". The current DAAs are therapeutically active while working at different stages of the virus life cycle by only targeting and inhibiting hepatitis C nonstructural proteins that are potentially involved in virus replication and translation [1]. Current DAAs for the treatment of hepatitis C target 3 nonstructural proteins of HCV upon which they are classified accordingly as shown in Fig. (**1**). As of August 2020, the following DAAs are no longer used in the USA for the treatment of HCV; boceprevir, daclatasvir, dasabuvir, elbasvir, grazoprevir, ombitasvir, paritaprevir, simeprevir, and telaprevir. Similarly, telaprevir and boceprevir are obsolete and no longer recommended in the rest of the world to treat HCV, but the remaining DAAs are prescribed by clinicians to treat hepatitis C [5]. Second, DAAs are only effective to achieve high SVR rates or cure hepatitis C when administered in combinations within a similar or different class or as a fixed-dose combination (FDC) of different DAAs as a single co-formulated pill [1]. Fig. (**2**) depicts the most promising direct-acting antivirals according to their classification while Table **1** demonstrates the salient features of the FDA-approved interferon-free DAA combinations for hepatitis C treatment.

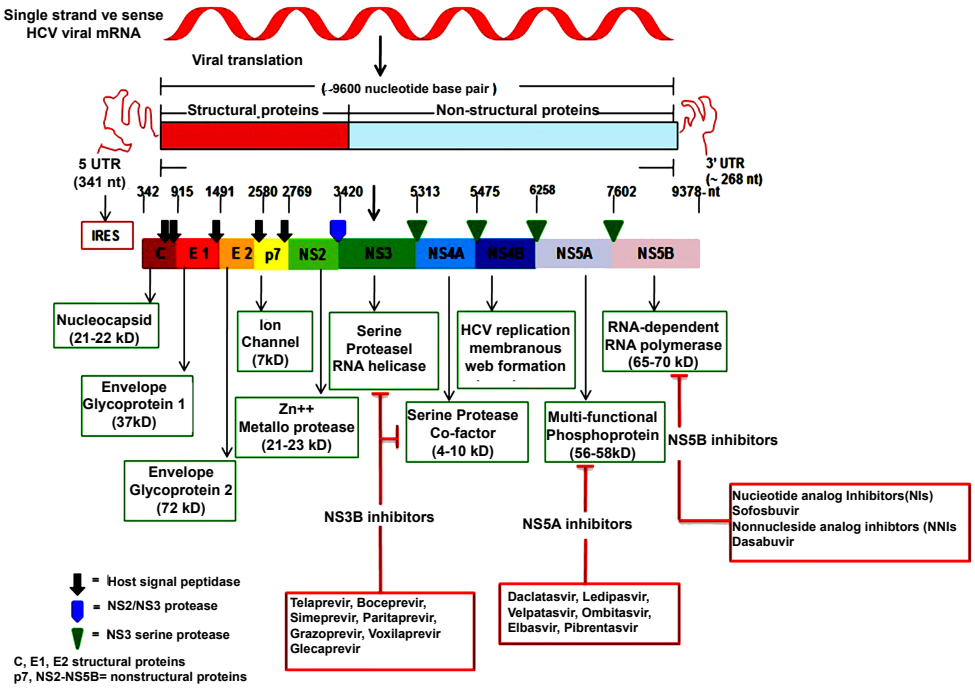

Fig. (1). Direct-acting antivirals acting on their targeted sites (highlighted in rectangular boxes) of the HCV genome UTR; untranslated region.

MECHANISM OF ACTION OF DAAS

As previously described, an ample understanding of the HCV genome and its life cycle enabled efforts to improve drug efficacy and tolerability to cure the infection [1]. The early treatment in the form of pegylated interferon (PEG-IFN) plus ribavirin (RBV) could not seem highly efficacious against HCV GTs 3 and 4 and their subtypes [2]. This led to the development of multiple direct-acting antivirals (DAAs) which target specific proteins pivotal for the HCV life cycle [1]. DAAs are drug molecules that target specific nonstructural proteins of HCV and disrupt viral replication, translation, and ultimately the infection [1]. There are four main classes of DAAs defined by their mechanism of action and therapeutic target. The four classes include;

1- Nonstructural protein 3/4A (NS3/4A) protease inhibitors (PIs)

2- NS5B nucleoside polymerase inhibitors (NPIs)

3- NS5B non-nucleoside polymerase inhibitors (NNPIs)

4- NS5A inhibitors

CHAPTER 7

Consensus Treatment Guidelines and Recommendations to Treat Hepatitis-C Infected Populations

Abstract: Unlike other infectious diseases and viral infections, the long-term chronicity of hepatitis C infection could worsen or propagate to irreversible extra-hepatic manifestations like decompensated cirrhosis or the development of hepatocellular carcinoma. The recent real-world clinical data of hepatitis C patients treated with IFN-free DAAs are still fewer to conclude or decide the best treatment protocols and guidelines for those who are still awaiting the treatment. However; based on the clinical data retrieved from the diverse patient cohorts, multicenter and multinational clinical studies, and pre- and post-therapeutic monitoring of hepatitis C-treated patients enable the clinicians, physicians, and health care providers to sketch consensus treatment guidelines and recommendations for the safe administration of DAAs in general and vulnerable hepatitis C infected populations. Interestingly and luckily, the treatment guidelines and recommendations approved by the FDA and CDC are following and working well in real-world clinical, hospital, and primary health care centers to manage hepatitis C, infected individuals. Albeit; for certain special populations like pediatric and pregnant hepatitis C females, we do not have clear guidelines for DAAs usage and their therapeutic monitoring. Furthermore, certain DAAs are not recommended in decompensated cirrhotics, in HCV rebound patients, and in previous treatment failure with a DAAs regimen. In this book chapter, we enlist updated treatment guidelines and recommendations to treat general as well as special hepatitis C-infected populations with DAAs and will briefly portray an overview of the pros and cons of these recommendations in real-world clinical settings.

Keywords: HBV reactivation, Hepatic fibrosis, Hepatic cirrhosis, HCV coinfection, Liver enzymes management, Post-treatment care, Pre-treatment history, RBV dose adjustment, Treatment monitoring, Treatment efficacy, Treatment referral.

INTRODUCTION

Many lessons have been learned from the current ongoing Covid-19 pandemic for the healthcare system, policymakers, research institutes, pharmaceutical industry, and academia of every country about the diagnosis, treatment, and management of

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an unprecedented pandemic outbreak at any time in the future [1]. Unfortunately, the devastating impacts of the pandemic both on economies and health sector programs of every country are still wearing on and we do not know how long the pandemic will last [1]. It has also highlighted the urgent need to minimize the complex cascade of diagnosis and care for complicated and critical-care patients of other endemic, epidemics, or syndemic infections all around the world (Fig. **1**). Furthermore, up-to-date clinical practice guidelines to reduce pandemic transmission as well as to ensure optimized patient treatment outcomes need to continue to evolve and be upgraded [1]. Rational to Hepatitis C, 71.1 million people are infected with this silent epidemic worldwide which corresponds to 1.0% of global prevalence [2]. Generally, as well as in the current situation of the Covid-19 pandemic, simplification of HCV diagnosis and particular treatment or care cascade pathways are urgently required to minimize the effect of the Covid-19 pandemic on the outcomes of patients with hepatitis c [3]. Furthermore, HCV clinical practice guidelines have a key role in guiding how to simplify the treatment or cascade of care for hepatitis C infected patients based on available data from clinical trials as well as from real-world cohort studies (Fig. **1**) [4]. Furthermore, guidelines also drive the pathways to eliminate HCV by advocating for simple hepatitis C care cascades as a part of recent national and international policies. Despite the significant advancement and remarkable progress toward simplified diagnostic and treatment algorithms, there remain some gaps to fill both in diagnostic and treatment strategies of HCV and to remove complexity in the recommendations for harder-to-cure, vulnerable, and marginal HCV-affected populations. Therefore, up-to-date and consensus HCV treatment guidelines are in eager demand, will always remain a priority, and is a continuous process to evolve to ensure HCV elimination [5].

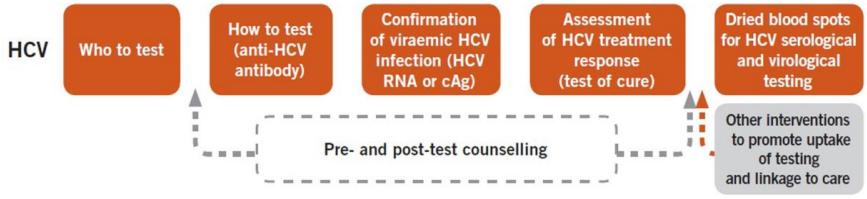

Fig. (1). A schematic diagram deciphering the importance of screening and treatment guidelines and pre-/post-test counseling for HCV patients [6]. HCV diagnostic and treatment guidelines play key roles in integrating HCV diagnosis and linkage to care. It may also be vital to promote the uptake of infection testing and enhance linkage to care. These guidelines may be very effective to bring more people to HCV testing and treatment while implementing pre-and post-test counseling.

Herein, we highlight consensus statements and current guidelines to consider for HCV treatment initiation decisions, monitoring during or after DAA treatment, post-treatment care, and post-treatment monitoring. Pre-treatment assessment of HCV-infected patients before recommendations for DAA administration is not a

part of this book chapter, as already described in detail in chapter 4. Furthermore, we will not point out here recommendations of DAAs for general and vulnerable HCV-infected populations as already discussed in detail in chapters 5 and 6 respectively, however; we will only focus on special guidelines or considerations while administering DAAs in HCV-affected populations.

Strength of Recommendation	**Quality of Supporting Evidence**
A = Strong	1 = At least 1 randomized trial with clinical outcomes and/or validated laboratory endpoints.
B = Moderate	2 = One or more well-designed, nonrandomized trial or observational cohort studies with long-term clinical outcomes.
C = Optional	3 = Expert opinion

MEDICAL HISTORY AND PHYSICAL EXAMINATION

The patient's demographics, triage, sign, and symptoms are essential components of pretreatment assessment before setting a criterion to accept for hepatitis C treatment initiation (Table **1**). Enlists here key elements of the patient history and physical examination that may apply specifically to the pretreatment assessment of CHC-infected patients [7].

Table 1. Key elements of patient demographics before initiating HCV treatment [7].

Key Elements of a Pre-HCV Treatment Patient History and Physical Examination	
Elements of Patient History	**Rationale**
Previous treatment for HCV infection	• Previous regimen and treatment outcome will guide the choice and duration of therapy.
History of hepatic decompensation	• Warrants referral to a liver disease specialist.
History of renal disease	• Findings may influence the choice of regimen.
Medication history and current medications, including over-the-counter and herbal products	• Carefully consider drug-drug interactions with DAAs. See the Drug-Drug Interactions section of this guideline.
Pregnancy status and plans (see the Pregnancy and HCV section of this guideline)	• HCV treatment is deferred during pregnancy. • Birth control use is essential during HCV treatment and for 6 months after treatment if patients are receiving ribavirin (RBV).

CHAPTER 8

Treatment Recommendations for Harder-to-Cure and Vulnerable Populations

Abstract: Certain hepatitis C-infected populations are still challenging to treat in the era of all-oral interferon-free direct-acting antivirals (IFN-free DAAs), which are highly efficacious, well-tolerable, and relatively safe in treated individuals. Such difficult-to-treat patients were also challenging even to manage with pegylated interferon (PEG-IFN) plus a nucleoside analog ribavirin (RBV) once known as the "gold standard of hepatitis C care". People infected with hepatitis C genotype 3, decompensated cirrhosis, individuals with co-infection status (*e.g.*, HCV/HBV, HCV/HIV, HCV/CKD), hepatitis C patients with induction of hepatocellular carcinoma (HCC), previous treatment failure with PEG-IFN plus RBV or DAAs failures, and viral relapse patients with the use of one or more DAA combinations are even compromised to achieve higher SVR rates with IFN-free DAAs. Similarly, some DAAs have sub-optimal clinical efficacies in harder-to-cure populations and some are contraindicated and can worsen hepatitis C-associated hepatic pathological states if administered without drug monitoring. Interestingly, DAAs in clinical trials conducted for their administration approvals demonstrated to achieve satisfactory SVRs in hepatitis C-infected special populations. Recently, limited data from real-world cohorts depict the excellent efficacy and safety of IFN-free DAAs in real-life clinical situations, similar to clinical trials. It is still uncertain whether either viral or host factors are responsible for the trivial effectiveness of DAAs in such populations. In this chapter, we will discuss the management of harder-to-treat special populations with DAAs by exploring some real-world cohort data as well as the treatment algorithms, guidelines, and recommendations for those patients in real-world clinical settings.

Keywords: Acute HCV treatment, DAAs for pediatrics, Decompensated cirrhosis, Hepatocellular carcinoma, HCV viremic organ, HCV/HBV coinfection, HCV/HIV coinfection, HCV/CKD coinfection, HCV correctional populations, HCV occupational treatment, Post-liver transplantation, Renal impairment, Renal transplantation, Regimens for retreatment, Substance use disorders, Treatment failure.

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INTRODUCTION

HCV Treatment in Patients with Compensated or Decompensated Cirrhosis

Chronic hepatitis C-infected individuals with cirrhosis have an increased risk of developing severe hepatic clinical manifestations, including hepatic decompensation, HCC, and death. Therefore, CHC-infected patients with compensated cirrhosis are on high priority to initiate treatment immediately after diagnosis [1, 2]. It means that an important step of pretreatment assessment for HCV patients is to determine whether the cirrhosis is compensated or decompensated. However, the treatment goals for CHC patients with decompensated cirrhosis need modifications if the patients are on the waiting list for liver transplantation [2].

Compensated Cirrhosis

HCV patients with compensated cirrhosis have mild hepatic impairment (*i.e.*, CTP class A) and do not have clinical hepatic manifestations of decompensated cirrhosis like jaundice, ascites, variceal hemorrhage, or hepatic encephalopathy [3].

Decompensated Cirrhosis

HCV patients with decompensated cirrhosis have a moderate or severe hepatic disease (*i.e.*, CTP class B or C, or a score of 7 or higher) and experience one or more of the following hepatic manifestations; ascites, jaundice, variceal hemorrhage, or hepatic encephalopathy [4]. HCV patients who show significant clinical improvement after experiencing one clinical feature of decompensated cirrhosis should be evaluated for DAAs treatment on a case-by-case to determine whether to initiate treatment similar to patients with compensated cirrhosis [2]. It is because of shreds of evidence that even hepatic decompensation patients recover from the acute event, they are still considered to have decompensated cirrhosis [5, 6].

HCV TREATMENT IN PATIENTS WITH COMPENSATED CIRRHOSIS

Multiple studies reveal that the treatment of HCV patients with compensated cirrhosis with IFN-free DAAs would significantly decrease the incidences of subsequent cirrhosis-related complications, including hepatic failure, HCC, and hepatic-related mortalities (Table **1**) [6 - 9]. The studies also reveal that the treatment of CHC in hepatic decompensation with DAAs also remarkably

improves the inflammatory grade of fibrosis following therapy and achieves higher SVR rates [10, 11]. Furthermore, it also substantially improves hepatic fibrosis following the achievement of an SVR with DAAs-based regimens [11].

Table 1. The available DAA options for HCV-infected patients with compensated cirrhosis [12].

HCV Genotype 1 population	Duration	Brands
Treatment naïve HCV GT-1/subtype 1a population:		
Glecaprevir 300 mg/pibrentasvir 120 mg once daily (A1)	8 weeks	GLE/PIB; (Mavyret®)
Ledipasvir 90 mg/sofosbuvir 400 mg once daily (A1)	12 weeks	LED/SOF; (multiple brands)
Sofosbuvir 400 mg/velpatasvir 100 mg once daily (A1)	12 weeks	SOF/VEL; (multiple brands)
Fixed-dose combination of elbasvir (50 mg)/grazoprevir (100 mg) one tablet once daily	12 weeks	(EBR/GZR); (Zepatier®)
Treatment naïve HCV GT-1/subtype 1b population:		
Glecaprevir 300 mg/pibrentasvir 120 mg once daily (A1)	8 weeks	GLE/PIB; (Mavyret®)
Ledipasvir 90 mg/sofosbuvir 400 mg once daily (A1)	12 weeks	LED/SOF; (multiple brands)
Sofosbuvir 400 mg/velpatasvir 100 mg once daily (A1)	12 weeks	SOF/VEL; (multiple brands)
HCV GT-2 population:		
Glecaprevir 300 mg/pibrentasvir 120 mg once daily (A1)	8 weeks	GLE/PIB; (Mavyret®)
Sofosbuvir 400 mg/velpatasvir 100 mg once daily (A1)	12 weeks	SOF/VEL; (multiple brands)
HCV GT-3 population:		
Glecaprevir 300 mg/pibrentasvir 120 mg once daily (A1)	8 weeks	GLE/PIB; (Mavyret®)
Sofosbuvir 400 mg/velpatasvir 100 mg once daily (A1)	12 weeks	SOF/VEL; (multiple brands)
• Sofosbuvir 400 mg/velpatasvir 100 mg one tablet once daily (A2); plus • Ribavirin 1000 mg if <75 kg or 1200 mg if ≥75 kg (the daily dose is given in two divided doses)	12 weeks	SOF/VEL; (multiple brands)
	12 weeks	RBV; (Copegus®)
Fixed-dose combination of sofosbuvir (400 mg)/velpatasvir (100 mg)/voxilaprevir (100 mg) one tablet once daily (A1);	12 weeks	SOF/VEL/VOX; (Vosevi®)

CHAPTER 9

Real-World Therapeutic Outcomes of Direct-Acting Antiviral Regimens and Formidable Challenges

Abstract: Oral interferon-free DAAs (IFN-free DAAs) have proven their clinical and therapeutic worth in real-life situations by achieving higher sustained virologic response rates (SVRs >90%) in treated individuals. After their recommendations to be administered to hepatitis C-infected populations in 2017 more than 5 million hepatitis C-infected individuals have been treated across the world and the overall health care burden of active hepatitis C comorbidities and mortalities have been declined from 130 million hepatitis C patients to approximately 71 million. Despite these great achievements in hepatitis C therapeutics, certain patient-oriented, clinical, and societal challenges are still prevailing to accept IFN-free DAAs on the large scale clinical, hospital, and primary health care settings in low and middle-income countries as well as even in developed nations. High therapy costs, treatment access and monitoring, co-infection status of certain vulnerable hepatitis C infected populations, racial disparity, pre-, and post-therapeutic monitoring, and long-term follow-ups are potential barriers to consensually implementing uniform treatment algorithms and accessibility to DAAs regimens worldwide. Furthermore, recurrence of hepatitis C infection, reactivation risks of co-infections (*e.g.*, HCV/HIV, HCV/HBV or HCV/CKD), minefield risks of hepatocellular carcinoma (HCC) rebound, and surveillance of hepatitis C liver transplant recipients which are on treatment with IFN-free DAAs also limit the administration of these regimens to every hepatitis C infected individual. In this book chapter, we will explore all these real-world challenges and will discuss/suggest the strategies to coup them in clinical, hospital, and community settings to improve the cascades of care and scale-up HCV cure.

Keywords: Clinical efficacy, Cascades of care, Dose algorithms, Direct-acting antivirals, Drug-drug interactions, Formidable challenges, HBV reactivation, Hepatocellular carcinoma, Resistance-associated substitutions, Resistance-associated variants, Real-world challenge, Retreatment, Sustained virologic response, Severe adverse events, Therapy cost, Treatment access, Treatment monitoring, Transplant recipients, Treatment failure, Viral fitness.

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INTRODUCTION

With the rapid advancement in HCV treatment by the advent and approvals of oral IFN-free DAAs in the last decade, clinicians have seen therapeutic outcomes they never thought possible in real-world clinical settings [1]. Furthermore, liver specialists and infectious disease experts are also cautiously optimistic that more harder-to-cure and challenging populations are also responding to DAA regimens [2]. Multiple studies from real-world clinical settings are deciphering the strong potential of pan-genotypic DAAs (Table **1**) to cure the general HCV-infected population as well as achieve higher SVR rates in difficult-to-treat fractions of HCV-infected individuals and among HCV patients with advanced hepatitis C associated comorbidities (*e.g.*, decompensated cirrhosis, hepatocellular carcinoma, *etc.*) [3 - 5]. The big picture on a clinic canvas in real-life situations reveals that 95 to 100% of patients treated for hepatitis C can be cured [5, 6]. Although, treatment choices can be tricky in certain classifications of HCV-infected populations; however, the end therapeutic outcome would be an SVR12 at the end of treatment either after 12 or 16 weeks, albeit, some clinicians monitor out to 24 weeks [7]. Interestingly, more than 99% of patients who achieved SVR rates will remain negative. Despite these higher SVR rates and significant cures of HCV in real-world clinical settings, the availability, accessibility, and affordability of DAAs regimens are still challenging in LMICs and even in resource-replete nations that prohibit treating everyone who is in need [4]. Furthermore, treatment-associated barriers in the form of pretreatment assessment, treatment monitoring during therapy, and post-treatment assessment/follow-ups are also influencing challenges to patient treatment adherence as well as to achieving successful SVR rates at post-treatment completion [4]. In addition to that, potential drug-drug interactions (DDIs) and DAAs incompatibility with certain other medications taken by HCV comorbid patients (*e.g.*, HCV/HBV or HCV/HIV or HCV/CKD) while treated by DAAs, as well as DAAs induced severe adverse events (*e.g.*, Abnormal ALTs, worsening of hepatic fibrosis or cirrhosis, HBV reactivation, and HCC recurrence), also pose hurdles to achieve hepatitis C cure in a large number of patients who receive active treatment for HCV [8]. Subsequently, HCV reinfections, virologic breakthrough, viral relapse, and treatment failure have also been demonstrated in general as well as harder-to-treat HCV individuals (*e.g.*, HCV GT 1a and 3 cirrhotic patients, MSM, IDUs, homeless HCV/HIV co-infection, and HCV infected liver transplant patients) with DAAs in real-world clinical settings [8]. Furthermore, DAA dosage algorithms and recommendations for administration to women of reproductive age (WORA), pregnant females, neonates, infants, children, and adolescents are not defined yet; although, various clinical trials are underway in this direction [9].

It is not possible in this book chapter to discuss real-world cohort studies in detail while covering every aspect of baseline HCV-infected patient demographics, DAAs treatment strategies, and outcomes of the treatment; however, we present some selected studies from real-world clinical experiences in tabulated form projecting DAAs therapeutic outcomes, efficacies, and safety in HCV infected populations as well as other comorbid conditions (Table **1**). We will also confine while discussing formidable challenges in HCV therapeutics that present potential barriers to overcoming the HCV healthcare burden in clinics, hospitals, and healthcare services providing centers as well as those hamper the efforts to eliminate this silent epidemic worldwide.

Table 1. The real-world therapeutic outcomes of DAAs against the hepatitis C virus in various parts of the world. T.N; treatment naïve, T.E; treatment-experienced, SVR; sustained virologic response, HIV; human immunodeficiency virus, GT; genotype, SOF; sofosbuvir, DCV; daclatasvir, LDV; ledipasvir, VEL; velpatasvir, RBV; ribavirin, EBR; elbasvir, GZR; grazoprevir; PrOD; paritaprevir, r; ritonavir, O; ombitasvir, D; dasabuvir, GLE; glecaprevir, PIB; pibrentasvir, VOX; voxilaprevir, RAS; resistance-associated substitution, PWID; patient who inject drugs, PEG-IFN; pegylated interferon, SMV; simeprevir.

Study (Year)	Study Country	Reference	Patient Demographics					Regimen	Treatment Duration (Week)	SVR12 (n/N)
			HIV (%)	Cirrhosis Status	PWID (%)	T.N/ T.E	GT			
1. Real-world effectiveness and safety of sofosbuvir and nonstructural protein 5A inhibitors for chronic hepatitis C genotype 1, 2, 3, 4, or 6: a multicentre cohort study [10]										
2020	Thailand	Phunchai Charatcharoenwitthaya *et al*.	2.1%	Compensated	-	T.N + T.E	1/2/3/4/6	SOF +DCV ± RBV	12/24	98.0%
								LDV/SOF±RBV		97.9%
								VEL/SOF ±RBV		96.5%
2. Efficacy and safety of ledipasvir/sofosbuvir in 5028 Mongolian patients infected with genotype 1 hepatitis C virus: A multicenter study [11]										
2020	Mongolia	Oidov Baatarkhuu *et al*.	-	Compensated	-	T.N + T.E	1	LDV/SOF	12	99.6% (5008/5028)
3. Treatment outcomes in hepatitis C virus genotype 1a infected patients with and without baseline NS5A resistance□associated substitutions [12]										
2020	Germany	Julia Dietz *et al*.	-	Compensated	-	T.N	1a	In the absence of RAS Overall SVR12:		
								EBR/GZR	12	97%
								LDV/SOF±RBV	12	99%
								GLE/PIB	12	99%
								PrOD + RBV	12	99%
								VEL/SOF	12	99%
								In the presence of RAS Overall SVR12:		
								LDV/SOF	12	100%
								GLE/PIB	12	83%
								PrOD + RBV	12	100%
								VEL/SOF	12	100%
								SMV/SOF	12	100%
								EBR/GZR+RBV	16	100%
4. Efficacy of direct-acting antivirals: UK real-world data from a well-characterized predominantly cirrhotic HCV cohort [13]										
2019	UK	Lucia macken *et al*.	-	Compensated	-	T.N + T.E	1	LDV/SOF ± RBV	12	Overall 91% (819/900)
								OBV/PTV/DSV/± RBV		
							3	SOF+ DCV + RBV	12	Overall 87% (396/455)
								SOF/PEG-IFN/RBV		

CHAPTER 10

Appling Drug Discovery in HCV-therapeutics: A snapshot from the past and glimpse into the future

Abstract: The ongoing COVID-19 pandemic with its devastating impacts in terms of huge disease burden and patient management on the world's leading healthcare systems and jolting the world's biggest economies, has leveraged the lesson that to prevent the transmission and elimination of a viral pandemic, endemic, or epidemic in future, a prophylactic or protective vaccine would be indispensable. In this scenario, DAAs regimens alone would not be sufficient to eliminate the HCV epidemic by 2030 or beyond and there would always be the demand for a prophylactic or protective vaccine to prevent the transmission of this epidemic again from vulnerable populations. The anti-mRNA-based treatment strategies (*e.g.*, anti-HCV protein-specific oligonucleotides, RNA interference (RNAi), and micro RNA (miRNA)), and some potential anti-hepatitis C vaccine models have been widely and extensively studied as an alternative or adjuvant therapeutic approaches for hepatitis C in the recent past and some of those models are still in the pipeline. The approval of the first RNAi therapy against a hereditary protein deposition disorder has urged investigators to refocus this approach against hepatitis C because it represents the most thoroughly studied treatment strategy against hepatitis C in the last two decades. Furthermore, some emerging approaches like host targeting agents (HTA), nanoparticles-containing immunogens, and nanomedicine-based therapeutic agents are also in their full investigative form. In this book chapter, we will discuss and highlight emerging hepatitis C treatment approaches that could be the game-changer to vanquishing HCV by 2030 while used as an adjuvant or compensatory regimen with DAAs.

Keywords: Antisense Oligonucleotides, Anti-HCV Vaccines, Controlled Infection Models, Distinct Conceptualization, Dnazymes, Emerging Therapeutics, Host Targeting Agents, Inorganic Nanoparticles, Micro-RNA, MiRNA Inhibitors, Monoclonal Antibodies, Nanozymes, Nanoparticle Immunogens, Nanomedicine, RNA Interference, Traditional Immunization Models.

INTRODUCTION

Since its initial discovery in 1989 through non-traditional virological techniques, remarkable progress has been made in hepatitis C biology, virology, immunology, diagnostics, and therapeutics. From that time until now, it would not be an exaggeration if we say that every notable discovery and invention in virology was

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tried to link hepatitis C research to comprehensively elucidating HCV infection cycle in host cells, generation of efficient *in vitro* cell culture systems, development of rapid molecular tests and simplified HCV screening and diagnostic algorithms to identify HCV infected persons, and the most important effective HCV therapeutics that can achieve SVR rates >95% of treated infections [1]. For example, in the last two decades, the world has never seen so much rapid advancement and revolution in diagnostics and therapeutics of any other infectious or contagious virus infection except the ongoing COVID-19 pandemic, where we have reached a point to eradicate this 'silent epidemic' with the help of the state-of-the-art IFN-free DAAs and some other emerging therapeutic paradigms in the late-stage of development [1]. This journey does not seem so easy and incredible success in HCV diagnostics and therapeutics involved the dedicated and untired contributions of so many famous and renowned investigators and scientists around the world. Subsequently, the participation and contributions of these efforts in HCV discovery and characterization were evident and admitted last year while awarding the Noble Prize in Medicine or Physiology to Micheal Houghton, Charles Rice, and Harvey Alter.

HCV DIAGNOSIS AND DRUG DISCOVERY-A CONTINUED JOURNEY

Despite these many successes while starting from interferon as an initial therapy to treat HCV-infected patients to promising pan-genotypic DAAs, which are highly efficacious with a cure rate of more than 95%, and transferring from initial HCV screening by a traditional ELISA test to digital self-test HCV diagnostic technologies, HCV research unfortunately yet could not succeed to deliver on one of its principles promises critical to achieving global elimination [2]. A key lack in HCV therapeutics is the deficiency of a prophylactic or protective vaccine. This failure is not due to a lack of trying, as multiple studies were carried out in the last 30 years to understand the kinetics of adaptive immune responses in the spontaneous clearance of acute HCV infection during 6 months in the majority of individuals and trying to translate this knowledge into the designing of a preventive vaccine [2]. Although; this knowledge was quite useful to understand the fundamental insights of protective HCV immunity (*i.e.* role of T cells) to achieve virus clearance, however, still not converted or transformed to develop an effective anti-HCV vaccine [2]. Consequently, significant barriers hinder global elimination efforts in the absence of a preventive anti-HCV vaccine to eradicate HCV by the year 2030 [1]. As HCV disease progression from acute infection to chronic and ultimately to end-stage liver disease (*e.g.*, HCC; hepatic failure) takes years to decades to develop, therapeutic termination of infection with promising anti-HCV agents (*e.g.*, current DAAs) represent a conceptually feasible strategy to prevent and probably eliminate hepatitis C and associated comorbidities [3]. However, it also seems an uphill task despite the remarkable efficacy of DAA

treatment because of their existing limitations such as difficult access to these expensive regimens in LMICs and resource-poor nations, the emergence of RASs, and DAAs associated with high risks of developing or recurrence of HCC in treated individuals [1]. Furthermore, inadequate infection surveillance programs, poor treatment adherence within harder-to-treat subpopulations, and the fear of remaining immunologically susceptible to reinfection even cured with pan-genotypic DAAs also complicate the elimination of HCV from the vulnerable fraction of populations (*e.g.*, IDUs, sex workers, MSM, opiate use disorders, and illegal immigrants with HCV/HIV co-infection) [1].

ANTI-MRNA BASED TREATMENT STRATEGIES

In the postgenomic era, the knowledge of the physiological functions of the genome has become the rate-limiting step in the quest to develop "gene-based therapies" [4]. The discovery of RNA interference (RNAi) technology known as once," one of the most exciting discoveries in biology in the last couple of decades", and 'breakthrough of the year' became rapidly one of the key methods in functional genomics and played a pivotal role in the validation of novel 'gene-based drugs' [5]. RNA interference (RNAi) is a fundamental cellular mechanism for silencing gene expression through a sequence-specific double-strand RNA degradation process to inhibit genome replication and treat various diseases. Since its discovery, the technology has been widely used to develop RNAi-based drugs (*e.g.*, small interference RNA (siRNA) and short hairpin RNA (shRNA)) to treat and prevent various human diseases like tumors, metabolic disorders, genetic diseases, and certain viral infections [5]. Furthermore, the mechanistic approach of this technology to be more effective while knocking-out gene functions in the cell cytoplasm, makes it the most gene-based therapy studied against HCV replication and translation inhibition in both *in vitro* and *in vivo* cell culture and animal models [5]. However, certain crosscutting barriers related to effective siRNA delivery to their target hepatic cells, siRNA toxicities, and their potential impact on immune systems prevented this technology from being effectively tested in humans. Recently, the approval of the first siRNA-based treatment (Patisiran: ONPATTRO™) by the US FDA against a hereditary protein deposition disorder (*i.e.* hereditary transthyretin-mediated amyloidosis) has sparked the researchers to refocus this approach against hepatitis C [6].

Like RNAi, a similar discovery of another small RNA, *i.e.* microRNA (miRNA) has also revolutionized our understanding of gene regulation while studying their pivotal roles as an important regulator in human development, cellular differentiation, adaptation to the environment, oncogenesis, and host cell interactions with pathogens [7]. Furthermore, the development of methods to measure and regulate microRNA activities also offer a new set of tools to be used

CHAPTER 11

Global Health Sector Strategy (2016-2021) Toward Ending Hepatitis-C: Promises, Policies, and Progress

Abstract: The worldwide prevalence of the hepatitis C virus takes a heavy toll on lives, communities, and health systems. Every year more than 4 million peoples die from hepatitis C-related liver cancers and cirrhosis- a mortality tool comparable to that of HIV and tuberculosis. It needs for a global health sector strategy stems from the scale and complexity of the hepatitis C epidemic, along with growing recognition of its massive public health burden, and the huge opportunities for action. It is a golden time now to establish a coherent public health hepatitis C response that prioritizes effective interventions, promotes service delivery approaches that ensure quality and equity to test and treat every hepatitis C infected individual, takes programs to achieve the sustained impact of hepatitis C diagnostics and therapeutics at the population level, and establishes clear stakeholder responsibility and accountability. There are unprecedented opportunities to act while ending the hepatitis C epidemic and are feasible with the tools and approaches currently available and in the pipeline. For the greatest impact, these opportunities should be combined and tailored for specific populations, locations, and settings. New opportunities and health sector policies provide a ray of hope for the elimination of hepatitis C as a public health threat. In this book chapter, we will highlight the goals, aims, opportunities, and barriers to a coherent public health policy for hepatitis C effective interventions at screening, diagnostic, and therapeutic scale in general and vulnerable hepatitis C infected populations and their consensus implementations to healthcare systems.

Keywords: Cost-effective analysis, Cohesive health policies, Delivery approaches, Delivering for equity, Focused action, Financing for sustainability, GHSS 2016-2021, Healthcare systems, HCV elimination goal, HCV research development, Innovation for acceleration, Priority actions, Progress status, Policy impact, Progress toward impact, Primary care program, Service coverage targets, Strategy implementation, Strategic directions, WHO progress.

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INTRODUCTION

"Human immunodeficiency virus (HIV), viral hepatitis B and C, and sexually transmitted infections (STIs) are major public health threats worldwide [1]".All these infections account for more than 2.3 million mortalities per year, which represents 14% of all deaths from infectious and parasitic infections, digestive diseases, and cancer [2]. An estimated 1 million new individuals are being reported per day infected with these infections and 1.2 million among those are developing cancer each year. New data estimates that 9.4 million people are receiving DAAs treatment to cure CHC infection, an almost 10-fold increase from the baseline of 1 million at the end of 2015 and before the establishment of the global health sector strategy GHSS; 2016-2021) that defines the goals, objectives, strategies, and their implementation to achieve HCV elimination by 2030 [3]. This scale-up of HCV treatment has been proven sufficient to reverse the trend of increasing mortality from HCV infection from the first time. For example, Egypt and Georgia, the two bright examples to follow and implement universal HCV diagnosis and access to treatment strategies defined by GHSS have resulted in declining mortality rates and new infection incidences [3]. However, improved data both in terms of viral screening and treatment access, indicate that the number of people dying from hepatitis B and C worldwide remains daunting at 1.1 million per year [4]. Interestingly, these communicable infections share common modes of transmission and determinants and call for a common public health approach along the continuum of prevention, diagnosis, treatment, and care. However, key differences exist in their status in 2021, with important lessons learned to design policies and strategies and to find opportunities for their implementation to achieve universal health coverage [2, 5].

In 2016, WHO member states established three aligned global health sector strategies on HIV, viral hepatitis, and STIs, respectively, to guide actions over the period 2016-2021 (*i.e.* GHSS 2016-2021) towards eliminating them as public health threats by 2030 [6 - 8]. The strategies share a common goal to progress towards universal health coverage and the 2030 agendas for sustainable development. Furthermore, the strategies were organized around a common framework that promoted synergy across the diseases and with other programmatic health priorities. At that time, the pace of progress was uneven and insufficient to meet global targets by 2030. The global response to hepatitis C was gaining momentum, but access to testing and treatment remained far below needs, and mortality was increasing. "Key and vulnerable populations were not adequately reached with essential interventions, and many opportunities for joint action were being missed. The report called for accelerating progress with integrated and people-centered approaches to end epidemics as public health threats by 2030 [8]".

THE MAIN GOALS OF GHSS (2016-2021) AND MAJOR GAPS

"The GHSS (2016-2021) covered the first six years of the post-2015 health agenda, building on the prevention and control of viral hepatitis infection along with the framework for global action on two resolutions of viral hepatitis adopted by the world health Assembly in 2010 and 2014 respectively [8]". The strategy addressed all five hepatitis viruses (hepatitis A, B, C, D, and E), with a particular focus on hepatitis B and C, owing to the relative public health burden they represent. One of the main goals of this strategy describes the elimination of viral hepatitis C as a major public health threat by 2030. For this purpose, short and long terms targets were set for 2020 and 2030 along with potential pragmatic strategies to achieve these targets [8]. As the implementation period for the GHSS for HIV, viral hepatitis, and STIs ended in 2021, it is the time of accountability for the main achievements and gaps to date and to highlight actions to take forward toward eliminating these diseases as public health threats by 2030 [8].

The estimates of a recent progress report published by WHO indicate that if we lose focus now, the progress achieved so far will plateau with the risk of resurgence [8]. According to the report, many gaps need to fill, most global 2020 targets have been missed, and accelerated actions are needed to reach the sustainable development goals in the next decade. For example, since the adaptation and implementation of GHSS 2016-2021, 21% more people infected with HCV infection have been diagnosed and 62% of those diagnosed receive treatment [5]. The price reduction has made HCV treatment an affordable high-impact intervention, but coverage needs to increase nearly sixfold in the next decade to reach the 2030 targets for elimination [9, 10]. Furthermore, Key and vulnerable populations were not adequately reached with essential interventions (*e.g.*, HCV screening and diagnosis), and many opportunities for joint action (POC HCV testing and treatment) were missed. Many countries are organizing strategies and planning frameworks to eradicate HCV; however, most are missing important opportunities to integrate and link services and responses to provide people-centered services that also leverage efficiency at the primary healthcare and health system levels [8, 9].

This book chapter overviews the main achievements since the establishment of GHSS 2016-2021toward ending viral hepatitis (in particular HCV) by 2030, addressing specific gaps in implementation, and bringing innovation to scale up HCV diagnosis, linkage to care, and the cascade of care to get the ambitious goal of HCV elimination worldwide. Furthermore, it provides new estimates on the disease burden of hepatitis C, which will enable a more informed global response as part of the next phase of strategies for the period 2022-2030 [10].

CHAPTER 12

WHO Hepatitis C Elimination Goal by 2030: Feasible or not?

Abstract: To put an end to hepatitis C from the world, the quality and equity of hepatitis C screening, diagnosis, and treatment must be accessible to everyone infected with the virus, regardless of age, sex, racism, nationalism, and religious differences. If several key strategies are successfully implemented, countries could collectively meet the WHO target of reducing new HCV infections by around 80% by 2030, compared with 2015. But even with successful implementation, the target of reducing HCV mortality by 65% would take until 2032, according to recent data. To evaluate the power of several interventions those help to reach these goals, several transmission models with data from affected countries that comprise hepatitis C patients demographics, virus prevalence in vulnerable populations, current dynamics of prevention programs, the natural history of hepatitis C and its prevalence, and percentages of deaths caused by hepatitis C must be considered. In addition to that, the models to project what it would take to reach the targets would need to change and improve blood safety and infection control, vertical transmission of hepatitis C infection, extending harm reduction services for PWIDs, expanded testing, and increased treatment with DAAs, with intensive improvements in public health care sectors and strong political will in third-world countries where hepatitis C is almost endemic would be required. In this book chapter, we are focusing on the achievements of the GHSS 2016-2021 plan for hepatitis C with their probable implementations in WHO member states as well as cross-cutting priority actions for the next decade.

Keywords: Community engagement, Diagnostic burnout, Digital health, HCV self-testing, HCV micro-elimination model, HCV macro-elimination model, Hepatitis C elimination, Linkage to care, Monitor outcome, Mobile diagnostic units, National framework, Potential program integration, Reflex HCV testing, Scale-up HCV testing, Task-shifting, Universal DAAs coverage, Virus surveillance.

INTRODUCTION

Hepatitis C remains a deadly viral infection since its discovery in 1989 to yet, although the DAAs breakthrough and plausible achievements of WHO viral hepatitis elimination targets by implementing the recommendations of GHSS 2016-2021 on viral hepatitis in member states have raised the hopes to eliminate

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this silent epidemic by 2030 or beyond [1]. As described earlier in chapter 11, although much progress has been made in distinctive areas of HCV screening, diagnosis, treatment, and monitoring in the last decade, many countries are still off-track to implement these tools, strategies, and policies in their national healthcare plan to curb HCV in the next decade. Consequently, an estimated 58 million people are still living with CHC and only 9.8 million get treatment since the approval of breakthrough all-oral IFN-free DAAs to treat all HCV GTs/subtypes infected patients. Furthermore, the distant obstructions while considering in the context of the ongoing Covid-19 pandemic also outpaced HCV diagnosis to find missing million from vulnerable HCV populations as well as to provide treatment who is in need [1]. The pandemic has also interrupted screening campaigns and routine health visits that confirm HCV diagnoses and identify new cases, as part of efforts to reduce people's risk of exposure to the SARS-CoV-2 virus. Indeed, the global viral hepatitis C response has been profoundly impacted by the Covid-19 pandemic [2]. The pandemic has not only paused HCV community outreach, education, screening, and linkage to care programs, but the lack of personal protective equipment (PPE), overwhelmed health systems, and looming austerity budgets threaten to roll back even the limited progress achieved since the debut of the HCV cure. The syndemic of blood-borne infectious disease, substance misuse, and overdose continues to take lives and shows signs of worsening as a result of the Covid-19 crisis [1]. Even before the pandemic shuttered economies across the globe, the HCV response had been woefully underfunded, with less than 10 percent of needs met. Political apathy, the challenges of mobilizing highly marginalized and criminalized communities for advocacy, limited licenses for affordable generics that exclude LMICs, and slow registration for generic production by both originator companies and national governments continue to be barriers to expanded access and scale-up. While our understanding of Covid-19 continues to evolve, as of press time, elevated liver enzymes have been observed among people who have recovered from Covid-19 infection [1]. This highlights the ongoing, and often unmet, need for life-long liver health and function monitoring for all those with current or past viral hepatitis C, cirrhosis, or other hepatic conditions [1].

Despite the expanded rollout of generic, curative treatments with pangenotypic DAAs, political apathy, less than 10% of the necessary funding, and diagnostics barriers have been sabotaging efforts to meet global HCV elimination targets [2, 3]. Enormous amounts of public funding and health resources have been reallocated to respond to Covid-19 around the world, compounding the complications of providing essential services, let alone scaling up the hepatitis C response [1 - 4]. Subsequently, staggering rates of new infections continue to outpace annual cures, and low treatment uptake and high mortality derail efforts to meet WHO 2030 targets [1, 4]. Of course, to achieve WHO set global targets to

eliminate HCV, diagnosis of new HCV infections is crucial for early detection and treatment and to prevent onward transmission of the virus. Chapter 11 of the book comprehensively discusses the framework of GHSS 2016-2021 on HCV and its achievements with cross-cutting barriers to overcome in this decade to smoothly achieve HCV elimination targets by 2030. Fig. (**1**) illustrates the continuum of viral hepatitis services and the retention cascade in an ideal healthcare system. Modeled studies predict that we need to treat 5 million people every year, worldwide, to achieve HCV elimination by 2030. However, different disease modeling suggests that only 11 countries are on track to achieving elimination by 2030 which are treating more than five patients for every new infection, and countries with higher cure rates for new infections (5:1 or more) are projected to achieve elimination by 2030 or sooner [1 - 4].

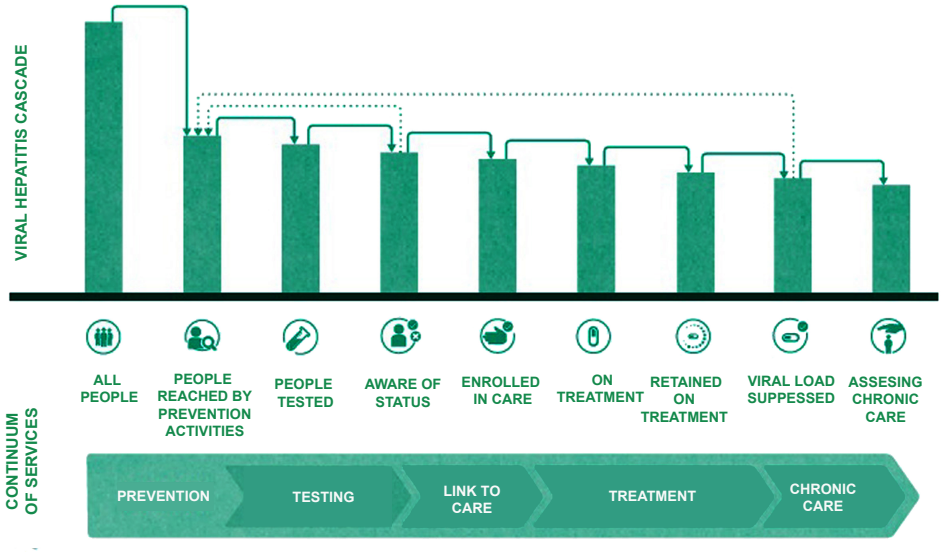

Fig. (1). The continuum of viral hepatitis services and the retention cascade [5].

To reach the 2030 targets, we need to accelerate progress, address specific gaps in implementation, and bring innovation in strategies to scale-up HCV diagnosis and treatment in this decade [1, 2]. Fig. (**2**) depicts the key measures to adopt while achieving both macro and micro-elimination of hepatitis C worldwide. Furthermore, barriers to diagnostics and treatment are also needed to overcome while identifying those and discussing strategies according to the available resources and considering their local and international contexts as they differ in each country. Social determinants of health (*e.g.*, stigma, racial disparity, marginalization, and criminalization) are also needed to end which prevents newly

APPENDICES

APPENDIX A

ALP alkaline phosphatase

ALT alanine aminotransferase

ANC antenatal clinic

APRI aminotransferase/platelet ratio index

ART antiretroviral therapy

ARV antiretroviral (drug)

AST aspartate aminotransferase

CDC U.S. Centers for Disease Control and Prevention

CHB chronic hepatitis B

CI confidence interval

CLIA chemiluminescence immunoassay

CrCl creatinine clearance

DAA direct-acting antiviral (drug)

DALY disability-adjusted life year

DBS dried blood spot (specimen)

ECL electrochemiluminescence immunoassay

eGFR estimated glomerular filtration rate

EIA enzyme immunoassay

ELISA enzyme-linked immunosorbent assay

FBC full blood count

FDA U.S. Food and Drug Administration

FIB-4 fibrosis-4 score

GDP gross domestic product

gGT gamma glutamyl transpeptidase

GHTF Global Harmonization Task Force

GHSS Global Health Sector Strategy

GRADE Grading of Recommendations Assessment,

HBcAg hepatitis B core antigen

HBeAg hepatitis B e antigen

HBIG hepatitis B immunoglobulin

HBsAg hepatitis B surface antigen

HBV hepatitis B virus
HCC hepatocellular carcinoma
HCV hepatitis C virus
HCVcAg hepatitis C virus core antigen
HDV hepatitis D virus
HIC high-income country
HIV human immunodeficiency virus
ICER incremental cost-effectiveness ratio
IFN interferon
IQR interquartile range
IVD in vitro diagnostic (medical device)
LMICs low- and middle-income countries
LoD limit of detection
LY life year
M&E monitoring and evaluation
MSM men who have sex with men
MTCT mother-to-child transmission
NAT nucleic acid testing
NGO nongovernmental organization
NIT non-invasive test
NPV negative predictive value
NRTI nucleos(t)ide reverse transcriptase inhibitor
OR odds ratio
OST opioid substitution therapy
PCR polymerase chain reaction
PEG-IFN pegylated interferon
PICO population, intervention, comparison, outcomes
PITC provider-initiated testing and counseling
AIDS acquired immunodeficiency syndrome
CDC Centers for Disease Control and Prevention
COVID-19 coronavirus disease 2019
FDA U.S. Food and Drug Administration
IDU injection drug use
PMTCT prevention of mother-to-child transmission
PPV positive predictive value

PQ (WHO) prequalification
PWID people who inject drugs
QA quality assurance
QALY quality-adjusted life year
QC quality control
QI quality improvement
RBV ribavirin
RCT randomized controlled trial
RDT rapid diagnostic test
RNA ribonucleic acid
RR relative risk
SOP standard operating procedure
SSA sub-Saharan Africa
STI sexually transmitted infection
SVR sustained virological response
TDF tenofovir
ULN upper limit of normal
D+o/p/r dasabuvir + ombitasvir/paritaprevir/ritonavir
UNAIDS Joint United Nations Programme on HIV/AIDS
UNICEF United Nations Children's Fund
WHO GHP WHO Global Hepatitis Programme
NIH National Institutes of Health
OR odds ratio
OUD opioid use disorder
POC point-of-care
RFI Request for Information
RNA ribonucleic acid
SARS-CoV-2 severe acute respiratory syndrome coronavirus 2
SSP syringe services program
STD sexually transmitted disease
STI sexually transmitted infection
SUD substance use disorder
VA Veterans Affairs
WHO World Health Organization
UMIC upper-middle-income country

Ab Antibody

Ag Antigen

fmol/L femtomole(s) per liter

FS Fingerstick

IU/mL International unit per milliliter

LLoD Lower limit of detection

MSM Men who have sex with men

ND No data

OST Opioid substitution therapy

PCR Polymerase chain reaction

POC Point-of-care

PQ Prequalification (For HCV, PQ is approved for diagnosis only, not SVR as LLoD has not yet been determined).

R&D Research and development

SVR Sustained virological response

TK To come

uL unit of liquid volume equal to one millionth of a liter, or 1 mm3

VL Viral load

BBV Blood-borne virus

GP General practitioner

NSP Needle and syringe program

PEP Post-exposure prophylaxis

PHC Primary healthcare

PrEP Pre-exposure prophylaxis

TasP Treatment as prevention

APPENDIX B

Acceptability. The degree to which given intervention is acceptable to the target population in relation to the effect of an intervention.

Accessibility. The degree to which given intervention is accessible to the target population (availability of good health services within reasonable reach and when needed).

Audit: Quality improvement process that aims to improve the care of patients by reviewing practices against criteria such as existing policy or guidelines and modifying practices where necessary.

Campaign: Series of activities and efforts to increase awareness and/or to promote initiatives. Campaigns can be conducted nationally or locally. For HIV testing national campaigns generally focus on increasing awareness, while local campaigns generally advertise local testing activities.

Checkpoint: Community-based center for detection of HIV and other sexually transmitted infections targeted at gay men other men who have sex with men, and transgender women.

Clinical decision-making tool: Clinical decision-making uses a combination of experience knowledge, and assessment tools to make effective clinical decisions. Clinical decision-making tool for HBV, HCV, or HIV testing is any strategy that aids staff in deciding who should get tested, *e.g.* patient-completed risk-assessment questionnaire or reminding staff to offer to test.

Community-based testing services: Programmes and services that offer voluntary free, and anonymous HBV, HCV, and/or HIV testing outside formal health facilities designed to target specific communities. For this guidance, such services include the following:

Community-based drug and harm-reduction service facilities: Provide community-based testing in a fixed location and specifically target people who use drugs. Typically though not universally, they maintain a low threshold for attendance and adopt a client perspective on service provision to make user access as simple as possible. Services at these facilities may include needle and syringe exchange programs, opioid substitution therapy, and other forms of drug treatment, as well as additional services such as HBV, HCV, and HIV testing, health-promotion activities, and social services.

Community-based outreach activities: Generic term covering several types of service delivery in the community that does not occur at a fixed site. They include services provided by mobile units and vans street outreach by community health workers, and regular satellite services sited in community-based facilities. Outreach services are often able to reach people who are not in contact with other health services by targeting them where they live or places they access. Such services play important role in identifying their needs and referring them to community-based facilities or public healthcare services.

Community-based testing facilities: Provide client-initiated (voluntary) testing services at a fixed location outside formal health facilities. These sites may also provide additional services such as counseling and health promotion activities. Community-based testing sites in EU/EEA are mainly focused on MSM and typified by peer-driven services (*e.g.* checkpoints).

Comparative study: Study designed to compare two or more groups or interventions (*e.g.* types of testing offered or test timings); statistical measure often provided for comparison.

Cost-effectiveness: Cost-effectiveness analysis is an aide to decision making that measures the ratio of costs of a program or intervention to effects it has on a defined outcome. For HIV testing this is defined as the threshold for HIV screening associated with favorable cost-effectiveness ratios when undiagnosed HIV prevalence rates are ≥0.1%.

Emergency department: Treatment facility specializing in providing medical and/or surgical care to patients who present to hospital often in need of immediate/urgent care. Most are open access with no requirement for the referral.

Feasibility: The degree to which it is possible to implement an intervention in terms of time money, or other circumstances.

Homelessness: A homeless person is an individual without permanent housing who may live on the streets stay in a shelter, mission, single-room occupancy facility, abandoned building or vehicle or live in any other unstable or non-permanent situation.

Incremental cost-effectiveness ratio (ICER): Measure used in cost-effectiveness studies to represent the value of intervention compared against an alternative (comparator). ICER is calculated by dividing the difference in total costs of two interventions by the measure of health outcome *e.g.* quality-adjusted life-year (QALY, see below). ICER determines whether a new intervention is an efficient use of resources.

Indicator condition guided-testing: For HIV the testing approach where HIV tests are routinely offered to all patients presenting with AIDS-defining illness or HIV indicator condition including STI, malignant lymphoma, cervical/anal dysplasia or cancer, herpes zoster infection, hepatitis B or hepatitis C infection, ongoing mononucleosis-like illness, unexplained leukocytopenia or thrombocytopenia, and dermatitis/exanthema.

Inpatient department: Hospital department where patients stay while they receive treatment.

Integrated testing: Provision of testing for more than one infection at the same time. For example HIV testing may be provided alongside testing for infections such as hepatitis B, hepatitis C, STIs, or TB.

Key populations: Include both most at-risk and vulnerable groups of people in a given population.

Late presentation: Occurs when the person is tested and diagnosed too late to either prevent avoidable harm or for treatment to be fully effective. For hepatitis late presentation is defined as persons presenting for care with chronic hepatitis B and C and significant fibrosis (≥ F3 assessed by either APRI score > 1.5, FIB-4 > 3.25, Fibrotest > 0.59 or alternatively transient elastography (FibroScan) > 9.5 kPa or liver biopsy ≥ METAVIR stage F3) with no previous antiviral treatment [4]. For HIV, late presentation is defined as persons presenting for care with a CD4 count below 350 cells/mL or presenting with an AIDS-defining event regardless of CD4 cell count.

Lay provider: Person providing healthcare in a community setting trained to deliver specific services such as blood-borne testing services, but has not completed formal professional healthcare training.

Low-threshold service: Service that places few restrictions on access and adopts client perspective on service provision to make utilization as accessible as possible for users.

Migrants: Individuals who change their country of usual residence irrespective of the reason for migration or legal status. Generally a distinction is made between short-term or temporary migration, covering movements between three and 12 months, and long-term or permanent migration, referring to change of country of residence for one year or more. For this guidance, migrants are individuals who originate from a country of intermediate or high endemicity for HBV/HCV/HIV or belong to local migrant communities known to have a high prevalence or incidence of HBV/HCV/HIV.

Opt-out testing: Testing modality where patients are informed they will be tested as part of routine care but may decline testing by objecting to the test.

Outpatient department: Hospital department that diagnoses and treats patients without requiring an overnight stay.

Outreach: Type of health service that mobilizes health workers to provide services to a population away from the location where providers usually work.

Partner notification/contact tracing: Process where individuals potentially exposed to infection are informed of exposure and offered testing and other interventions dependent upon specific infection. When contact is of a sexual or injecting nature this process is also referred to as partner notification. Partner notification is a voluntary process in which a trained provider asks a person diagnosed with HBV, HCV, or HIV about their sexual partners, at-risk drug-injecting partners, and household contacts as appropriate for diagnosis. With the individual's consent, the provider then offers, facilitates, or provides advice on testing for relevant infections to these partners and contacts, as well as linking them to preventive interventions such as vaccination (HBV) or post-exposure prophylaxis (PEP) for HIV. The identity of a diagnosed person is not revealed to contact by the provider unless consent has been given to do so. Web-based partner notification is an approach delivered via websites that allow users to send emails, e-cards, or text messages to inform partners anonymously.

Post-exposure prophylaxis (PEP): Use of antiretroviral therapy following exposure to HIV infection to try to prevent the establishment of infection.

Prevalence: Prevalence measures the proportion of individuals in the defined population with a specific disease (or specific characteristic) at a certain point in time. High intermediate, and low prevalence rates may be defined for HCV, HBV, and HIV to guide testing strategies after taking local epidemiology and other circumstances into account. Present guidance applies the following definitions of prevalence rates based on several published thresholds:

• Intermediate HBV and HCV prevalence: When HBsAg seroprevalence or HCV antibody seroprevalence in the general population is between 2% and 5%. For both HBV and HCV the high prevalence is ≥5%.

• High HIV prevalence: When HIV prevalence consistently exceeds 1% in the general population.

Pre-exposure prophylaxis (PrEP): Antiretroviral therapy-based HIV prevention strategy to prevent or at least reduce risk of HIV infection in adults who have not been infected with a virus but are at high risk of infection.

Primary care: Healthcare provided by general practitioners (GPs) nurses, and ancillary healthcare workers and first point of contact for healthcare for the majority of the population.

Provider-initiated testing: Voluntary testing offered to eligible individuals by healthcare providers.

People who inject drugs (PWID): People who inject non-medically sanctioned psychotropic (or psychoactive) substances. These drugs include but are not limited to opioids amphetamine-type stimulants, cocaine, hypnosedatives, and hallucinogens. The injection may be through intravenous, intramuscular, subcutaneous, or other injectable routes.

Quality-adjusted life year (QALY): Composite measure of health adjusted to reflect length and quality of life. One QALY equates to one year of life in perfect health. For an individual requiring an intervention QALY would be the weighted value of each year remaining to the patient with a quality of life score (on a 0 to 1 scale). In cost-effectiveness studies, QALYs are used to assess the effectiveness of a new intervention against the baseline intervention.

Rapid diagnostic test (RDT): Test that provides the result with a short turnaround time typically with oral fluid or finger-prick blood sample. RDTs are employed for point-of-care rapid tests and self-testing.

Reflex testing: Occurs when a positive test automatically initiates performance of another test typically to improve diagnostic sensitivity or presence of active infection. For this document, reflex testing refers to performing HCV nucleic acid test (NAT) on the same sample as a positive antibody screening test to detect active HCV infection.

Retesting: People who should be retested after a defined period. This includes HIV HBV, and HCV-negative people with recent (to cover the window period) or ongoing risk of exposure and people with inconclusive HBV/HCV/HIV status.

Risk group testing: Testing strategy targeted at groups identified as being at higher risk of infection. Identification of these groups depends on local epidemiology and typically includes men who have sex with men (MSM) prisoners, sex workers, people who inject drugs (PWID), and migrants.

Self-sampling: When individual collects a blood or saliva sample from themselves typically outside a healthcare setting, using a suitable kit. The sample is then delivered to a designated laboratory for processing. Results are usually delivered by phone, text message, or online, with referral mechanisms in place to ensure linkage to treatment and care as appropriate.

Self-testing: When an individual collects a blood or saliva sample then uses a rapid diagnostic kit to process the sample, obtain results and interpret them according to instructions provided with the kit. The kit typically includes linkage information to care as appropriate.

Sex workers: Individuals who receive money or goods in exchange for sexual services and consciously define those activities as income generating even if they do not consider sex work to be their occupation.

Task sharing: Rational redistribution of tasks and increased scope of work among different cadres of healthcare providers including trained lay providers.

Testing coverage: Extent to which testing program covers potential need usually measured as the proportion of persons tested in a given population.

Testing uptake: Rate of acceptance of testing by individuals offered a test in a given population.

Testing strategy: Describes testing approach to attain a specific objective that takes into consideration prevalence in the population tested.

Traditional and non-traditional settings: For HIV testing traditional settings are specialist healthcare settings where HIV testing is provided, including dedicated STI and sexual health clinics, antenatal services, and infectious disease units. Non-traditional settings are non-specialist for HIV testing such as general practice, community settings, and hospital outpatient departments.

Trans* people: Trans is an overarching term referring to those people whose internal perception of their own gender (gender identity) and/or a gender expression differs from the sex they were assigned at birth. The term trans includes but is not limited to men and women with transsexual pasts and people who identify as transsexual transgender, transvestite/cross-dressing, androgyne, polygender, genderqueer, agender, gender variant, or with any other gender identity and/or expression that is not standard male or female and express their gender through their choice of clothes, presentation or body modifications, including undergoing multiple surgical procedures.

Treatment as prevention (TasP): The impact of antiretroviral therapy to reduce the HIV viral load to undetectable levels effectively preventing onward transmission.

Universal testing: Strategy of offering HIV tests to everyone regardless of individual risk. Settings where this strategy may be particularly relevant, include hospital departments and general practice. In certain settings, such as antenatal services, universal testing may be opt-out (see above).

Window period: For tests designed to detect a specific disease the window period is the between first exposure to infection and the point in time after when the test can give the definitive (accurate and reliable) result.

AASLD/IDSA: American Association for the Study of Liver Diseases/Infectious Diseases Society of America.

ActiTest: A noninvasive test to determine the extent of liver inflammation damage, and scarring; however, it is not widely available outside high-income countries. This test is assessed on FibroTest and must be analyzed using BioPredictive diagnostics services. The test was developed using French public funding, then granted exclusive licensing to FibroTest.

Acute Infection: A recently acquired infection which may have mild or no symptoms; acute HCV infection may result in some inflammation to the liver.

ALP: Alkaline phosphatase an important liver enzyme used in determining your liver health.

ALT; SGPT: Alanine aminotransferase or serum glutamic pyruvic transaminase liver enzymes used in determining your liver health.

Antibody: Part of a person's immune system that responds to viruses bacteria, and other harmful substances.

APRI: Aspartate aminotransferase (AST) to platelet ratio index a formula used to determine the level of liver disease.

ART: Antiretroviral therapy a combination of antiretroviral drugs to suppress HIV and to stop HIV from progressing.

ARV: Antiretroviral drugs in the context of HIV, these medications suppress viral activity by preventing the virus from reproducing.

Assay: A diagnostic test performed to determine the presence of an infectious disease (qualitative assay) and if present the amount (quantitative assay).

AST; SGOT: Aspartate aminotransferase or serum glutamic oxaloacetic transaminase a liver enzyme made in the heart, intestines, and muscle used in determining your liver health.

cAG: HCV core antigen the viral protein of HCV, which can be found in the bloodstream within two weeks of infection. This type of confirmation test detects whether a person is currently infected with the virus.

CD4: Cluster of differentiation 4 a type of protein found in immune cells such as T cells, a type of white blood cells that lead the fight against infections. HIV infects the CD4 cells and then uses them to make copies of the virus. The CD4 count test calculates the total white blood cells and the proportion of cells that are CD4 positive. CD4+ cell count is used to monitoring the health of the immune system and antiretroviral treatment success. If the count drops lower than 200 cells per millionth of a liter, the person is at higher risk of opportunistic infections. Current WHO treatment guidelines indicate a person should start treatment at the time of diagnosis, regardless of CD4+ cell count. This improves the health of the person living with HIV and helps reduce the amount of the virus in the blood (viral load) and other bodily fluids to undetectable levels, which prevents transmission.

CDC: Centers for Disease Control and Prevention the U.S. agency that develops and applies disease prevention and control programs, as well as environmental health, health promotion, and health education activities.

CE-marked: Conformité Européenne a certification mark that shows the compliance to safety, health, and environmental protections for products within the European Economic Area.

Chronic Infection: An infection that can last for weeks months, or a lifetime, causing more damage to the affected tissue or organs.

Cirrhosis: Serious liver scarring or damage that can lead to liver failure.

CKD: Chronic kidney disease a condition where the kidneys' ability to filter harmful waste and excess fluid from the blood deteriorates over time.

CL: Compulsory license a license granted by a governmental administrative or judicial body to a third party to manufacture or use a patented product, such as medication, without the consent of the patent holder. A CL is an effective strategy to enable generic competition and reduce the price of patented medicines. The the patent holder is remunerated such as through royalty payments.

Community: A form of self-organization of a group of people with one or several characteristics that unite people living in nearby locations, foster relations between them, and share culture and values within the scope of these relations. A community can be defined by the following people, location, and relationship.

DAA: Direct-acting antivirals all-oral, highly effective medications for treating hepatitis C in usually 8 or 12 weeks. People with cirrhosis or other conditions may need to be treated longer to achieve sustained virological response, or when the virus is undetectable. DAAs can achieve sustained virological response in over 95% of patients, and the latest regimens treat all genotypes of the virus with similar results.

DBS: Dried blood spot blood sample obtained by pricking the finger or heel that is preserved and dried on filter paper. DBS can be easily shipped from a remote testing site to a lab for analysis.

DCV: Daclatasvir (also abbreviated as DAC); a direct-acting antiviral that is taken with sofosbuvir once daily with or without food for 12 or 24 weeks.

Diagnostic Burnout: The phenomenon in which countries are treating mainly patients known to have HCV and are failing to diagnose new infections. In this case countries will run out of diagnosed patients to treat, HCV transmission will continue to proliferate, and new infections will go undiagnosed, leading to advanced liver disease and liver-related deaths.

EASL: European Association for the Study of the Liver.

EDL: Essential Diagnostics List WHO guidance and reference on the tests and medical devices needed to diagnose the most common conditions as well as a number of global priority diseases. The EDL helps countries decide on the type and categories of diagnostics depending on their epidemiology, testing strategies, human resources, and healthcare and laboratory infrastructure.

EIA: Enzyme immunoassay antibody tests used in labs that detect antibodies in blood samples.

ESLD: End-Stage Liver Disease chronic liver failure, often as a result of cirrhosis.

FDC: Fixed-dose combination two or more drugs contained in a single dosage form, such as a capsule or tablet.

FibroScan: An ultrasound machine for your liver that measures liver stiffness scarring, and damage as well as the accumulation of fat in the liver.

FibroTest: A noninvasive blood test that can measure the degree of liver damage in a person; it requires results to be analyzed by BioPredictive software.

FIB-4: An inexpensive noninvasive calculation to determine the amount of liver scarring by using patient's age, platelet count, AST, and ALT.

Fingerstick: Also known as fingerprick a method of collecting a small amount of blood by pricking a finger with a lancet, or a sharp needle-like instrument.

Genedrive: A portable point-of-care confirmatory test, using plasma, for the detection of HCV RNA. It is designed for use in low-resource settings and delivers results in 90 minutes.

Genotype: Abbreviated as GT different subtypes of HCV, or a way to put HCV into categories based on similar genes. HCV has six genotypes, labeled 1 through 6. There are also subtypes labeled with letters, for example, genotypes 1a and 1b. Genotypes respond differently to medicines that treat and cure HCV, but pangenotypic (all genotypes) DAAs have similar SVR rates for all genotypes.

GGT: Gamma-glutamyl transferase an important liver enzyme used in determining your liver health.

G/P: Glecaprevir and pibrentasvir DAA taken once daily, as three pills, with food, that treats all genotypes of HCV in 8 weeks for people with no cirrhosis, or 12 or 16 weeks for people with compensated cirrhosis or other complications. It has been approved by the FDA for children and adolescents aged 12 and older who weigh at least 99 pounds (44 kilograms).

HBIG: Hepatitis B immune globulin an injection used to protect against hepatitis B within 24 hours of exposure.

HBV: Hepatitis B virus a highly contagious virus that infects the liver transmitted through blood, semen, and vaginal fluid. You can protect yourself against hepatitis B by getting vaccinated or having an injection of HBIg within 24 hours of exposure.

HCC: Hepatocellular carcinoma liver cancer, occurring most often in people with chronic liver diseases.

HCV: Hepatitis C virus a virus transmitted through blood, although small amounts have been found in semen and vaginal fluid, that causes hepatitis C, a liver infection that causes inflammation and tissue damage. There is no preventative vaccine, but all-oral DAAs can now effectively cure HCV. Untreated HCV can lead to severe liver damage and liver cancer.

HIV Self Test A simple blood test that a person can use on themselves to test for HCV; expected to become available in 2020.

HICs: High-income countries.

HIV: Human immunodeficiency virus a virus that weakens the immune system, ultimately leading to acquired immunodeficiency syndrome (AIDS).

IL28B CC genotype: The IL28B gene is involved in the immune response to certain viruses, including HCV. There are three IL28B subtypes (genotypes) CC, CT, and TT. People with the CC genotype have a stronger immune response to HCV infection than people with the CT or TT genotypes. In the pegylated interferon treatment era, IL28B genotype determined the type—and possibly length—of HCV treatment.

INHSU: International Symposium on Hepatitis Care in Substance Users.

Invasive Test: Any type of medical test that requires the use of instrumentation to physically enter the body such as a liver biopsy.

IP: Intellectual property refers to the concept of ownership over an idea or design, in which the person or entity that developed that idea or design is given exclusive right over it for a period of time, usually 20 years under international agreements. No one can copy or reuse the idea or design without the owner's permission. Common forms of intellectual property include patents copyrights, trademarks, industrial design rights, geographical indications, and trade secrets. In international law, legal mechanisms, such as compulsory licenses and patent oppositions, exist to gain access and manufacturer technological advancements, such as medicines, in order to create less expensive, generic versions.

LGBTQ+: Lesbian, gay, bisexual, transgender, queer/ questioning, and others an umbrella acronym that encompasses a spectrum of gender and sexual identities but is not comprehensive as the terminology is evolving and changing.

LED: Ledipasvir a direct-acting antiviral that is taken with sofosbuvir as two drugs in one pill, taken daily, with or without food, for 8 to 24 weeks. It is used for people with genotypes 1, 4, 5, and 6 who are over 12 years old.

LFT: Liver function test a group of blood tests used to detect, evaluate, and monitor liver disease or damage.

LMIC: Low-and middle-income countries.

LoD: Limit of detection in chemistry, the smallest amount of a microorganism or genetic material from a virus in a sample that can be reliably detected in optimal conditions. LoD is a step in determining the sensitivity of a test.

MAT: Medication-assisted treatment which uses medications such as buprenorphine, methadone, and naltrexone, often combined with counseling and behavioral therapy to treat opioid use disorders also referred to as OST.

MIC: Middle-income country.

Mixed Infection: People who are infected with more than one HCV genotype. This is most likely to happen to people who received blood products or blood transfusions many years ago or in a place where the blood supply was not checked for HCV people on kidney dialysis, or people who inject drugs with shared unsterilized equipment.

MSM: Gay bisexual, and other men who have sex with men.

Multiplexing: Testing for more than one infection at the same time on one device such as HIV, HBV, and HCV.

NAT: Nucleic acid amplification test a highly sensitive laboratory process used to detect genetic material, such as RNA of HCV. The NAT uses repeated chemical reactions (polymerase chain reaction) to make multiple copies of the HCV RNA that is trying to be detected. In this way, it is easier to detect tens of thousands of copies of a gene than only a few copies.

ND: No data.

NHANES: National Health and Nutrition Examination Survey a survey conducted by the CDC assessing the health and nutritional status, including the hepatitis epidemiology of adults and children in the United States. There are limitations because it did not include homeless people, incarcerated people, nursing home residents, people living on Native American reservations, or active military personnel, and it did not show wide geographic representation. Information on members of additional racial and ethnic groups was classified as "other".

Noninvasive Test: Medical test that does not require a medical instrument to physically enter in the body such as using ultrasound.

NSEP: Needle syringe exchange programs.

OST: Opioid substitution therapy which uses medications such as buprenorphine, methadone, and naltrexone, is often combined with counseling and behavioral therapy to treat opioid use disorders also referred to as MAT.

Pangenotypic: DAA treatments that can effectively cure all HCV genotypes at nearly the same rates.

PCR: Polymerase chain reaction the process during a nucleic acid amplification test to detect genetic material, such as HCV RNA, by making multiple copies of the HCV RNA that is trying to be detected. The PCR HCV RNA test determines how much of the virus (or viral load) is in the bloodstream.

PEG-IFN: Pegylated interferon an older treatment regimen requiring regular injections, combined with oral doses of ribavirin, between 12 to 48 weeks; no longer recommended by WHO, AASLD/IDSA, or EASL. Countries without access to direct-acting antivirals or prisons may still be using pegylated interferon.

PLHIV: People living with HIV preferred to use the people-centered term instead of the abbreviation.

PoC: Point-of-care test diagnostic testing conducted at the time and place of patient care, such as community clinics, harm reduction sites, and prisons.

Polyvalency: One machine can do different tests but uses a different sample and not always at the same time.

PT: Prothrombin time measurement for how long it takes for blood to clot.

PWID: People who inject drugs preferred to use the people-centered term instead of the abbreviation.

Qualitative RNA: Viral load test that checks whether there is RNA genetic material such as from HCV, in the bloodstream. The test result is either positive (the virus is detectable) or negative (no virus can be detected).

Quantitative RNA: Viral load test that measures the amount of RNA genetic material such as from HCV, in the bloodstream. These tests, while not available in every country, are also used to monitor HCV treatment to see if it is working.

R&D: Research and development.

RBV: Ribavirin oral treatment for hepatitis C, given as pills or capsules, twice a day. The dose depends on a person's weight. Ribavirin can increase cure rates in people with cirrhosis and is sometimes added to DAAs, although it causes side effects, such as anemia, insomnia, fatigue, irritability, and depression. Ribavirin is not recommended for men and women who are planning a pregnancy because it causes birth defects. Male partners of women who become pregnant or are nursing should use condoms for six months after completing HCV treatment.

RDT: Rapid diagnostic test antibody tests designed for use at the point of care.

Reagent: Chemical ingredients that are added to test a patient's sample to see if a reaction occurs. A change in color intensity in light, or other differences may occur. The change is measured against a known change or control and then calculated for a test result.

Reagent cartridge: A container with prefilled reagents to be loaded inside a diagnostics device.

Reflex testing: When additional tests are automatically ordered after a specific test result. For HCV if a patient has a positive antibody test, confirmatory tests (HCV RNA or core antigen) would be automatically ordered.

Reinfection: When a person who has been successfully treated such as for HCV, becomes infected again. HCV RNA testing is recommended for people with ongoing behaviors that may put them at risk of HCV infection. Access to sterile injection equipment, OST, and comprehensive prevention and harm reduction services is necessary to prevent reinfection.

RVD: Ravidasvir (also abbreviated as RAV) taken with sofosbuvir as a generic regimen that is under study in clinical trials, which may be used to treat all genotypes. It is taken once daily for 12 weeks or 24 weeks for people with compensated cirrhosis.

Sensitivity: True positive rate a test's ability to correctly identify people with an infection, such as HCV. High sensitivity—closer to 100%—helps avoid missing an HCV infection and determines the quality of a test.

Serological tests: Also known as screening or antibody tests generally used as the first step in a testing strategy due to the relatively lower cost. These tests detect antibodies or a viral antigen, and which people may have an active infection. They can be conducted with rapid diagnostic tests or laboratory-based enzyme immunoassays.

Serum: Clear and watery part of the blood that separates when the blood clots or is centrifuged (spun at high speeds).

SOF: Sofosbuvir an HCV-fighting DAA that must be used with other drugs. In combination with other DAAs, it can treat all genotypes in patients 18 years old and older; sofosbuvir with or without ribavirin can be used to treat genotypes 2 and 3 in patients 12–17 years old (2018 WHO Guidelines). Sofosbuvir is taken once daily, with or without food, for 12 to 24 weeks, depending on a person's liver damage, other health conditions, and treatment experience.

Specificity: True negative rate the test's ability to correctly identify people without HCV. High specificity— closer to 100%—helps avoid misinforming a person about being infected when they are not and determines the quality of a test.

SRA: Stringent Regulatory Authority a quality assurance policy to ensure certain requirements are met at a manufacturing facility for medicines or medical devices. This includes a list of countries with a national drug regulatory authority that are members observers, or associates of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). The SRA aligns with the Global Fund Quality Assurance Policy for Pharmaceutical Products which took effect on July 1, 2009.

Steatosis: Accumulation of fat in the liver; sometimes referred to as fatty liver.

STI: Sexually transmitted infection.

SVR: Sustained virological response a person has no detectable HCV after treatment has been completed.

Throughput: Rate of production or amount, of tests that can be run through a diagnostics system at a time.

VEL: Velpatasvir combined with sofosbuvir as the fixed-dose combination that treats all HCV genotypes, taken once daily, with or without food, for 12 weeks. People who have advanced (or decompensated) cirrhosis will need to add ribavirin twice daily.

Virological testing: Also known as confirmatory testing uses NAT. Laboratory processes to detect genetic material (DNA or RNA) of a virus and determine if the infection is active and to decide whether to start a patient on treatment. It is also used to confirm sustained virological response (for HCV) or effective suppression of the virus (for HBV).

VL: Voluntary license a patent holder allows another pharmaceutical company to manufacture a generic version of their medication. The patent holder sets conditions and may receive a fee or royalty. In effect, voluntary licenses permit patent holders/originator companies to control the market by limiting the countries that are licensed to produce and sell generics. Countries that are not included in licensing agreements must buy more expensive drugs from the originator companies. Voluntary licenses can also include other restrictions, such as the number of people who can be treated, whether drugs can be coformulated, and which suppliers must be used for the active pharmaceutical ingredients needed to make the medication.

WHO PQ: World Health Organization Prequalification a process conducted by the WHO using unified standards to assess the quality, safety, and efficacy of medicines, active pharmaceutical ingredients, medical products, and quality control laboratories. It is not a prescriptive list; rather, this standard can help countries consider medicines and medical devices that meet high-quality standards to procure for their national programs.

HB surface antigen (HBsAg): HBV envelope protein often produced in excess and detectable in the blood in acute and chronic HBV infection.

HB core antigen (HBcAg): HBV core protein. The core protein is coated with HBsAg and therefore not found free in serum.

HB e antigen (HBeAg): Viral protein found in the high replicative phase of HBV. HBeAg is usually a marker of high levels of replication with wild-type viruses but is not essential for viral replication.

HB surface antibody (anti-HBs): Antibody to HBsAg. Develops in response to hepatitis B vaccination and during recovery from hepatitis B denoting past infection and immunity.

HB core antibody (anti-HBc): Antibody to HBV core (capsid) protein. Anti-HBc antibodies are non-neutralizing antibodies and are detected in both acute and chronic infections.

anti-HBc IgM: Subclass of anti-HBc. Detected in recent HBV infection but can be detected by sensitive assays in chronic HBV infection.

HBVe antibody (anti-HBe): Antibody to HBeAg. Detected in persons with lower levels of HBV replication but also in HBeAg-negative disease (i.e. HBV that does not express HBeAg).

HBV DNA: HBV viral genomes that can be detected and quantified in serum by nucleic acid testing (NAT).

Anti-HCV antibody: Antibody to HCV which can be detected in the blood usually within two or three months of HCV infection or exposure. The terms HCV antibody and anti-HCV antibody are equivalent, but in the guidelines of this book, both are used throughout.

HCV RNA: HCV viral genomes that can be detected and quantified in serum by nucleic acid testing (NAT).

HCV core antigen (HCVcAg): Nucleocapsid peptide 22 [p22] of HCV which is released into plasma during viral assembly and can be detected from early on and throughout the course of infection.

Chronic HBV infection: Persistence of HBsAg for at least six months. The persistence of HBsAg in two specimens six months apart is frequently used in clinical practice to confirm chronic hepatitis B infection.

Chronic HCV infection: The presence of viraemic HCV RNA or HCVcAg in association with positive serology for HCV antibody.

Viraemic infection: Hepatitis B or C infection associated with the presence of virus in the blood (as measured by HBV DNA or HCV RNA) and often referred to as active, ongoing, or current infection.

Occult HBV infection: HBsAg negative but HBV DNA positive although at very low levels (invariably <200 IU/mL). Most are also anti-HBc positive.

Cirrhosis: An advanced stage of liver disease characterized by extensive hepatic fibrosis nodularity of the liver, alteration of liver architecture, and disrupted hepatic circulation.

Decompensated cirrhosis: Clinical features are portal hypertension (ascites, variceal hemorrhage, and hepatic encephalopathy) coagulopathy, or liver insufficiency (jaundice). Other clinical features of advanced liver disease/cirrhosis may include hepatomegaly, splenomegaly, pruritus, fatigue, arthralgia, palmar erythema, and edema.

Hepatocellular carcinoma (HCC): Primary cancer of the liver arising from the hepatocytes and may be a complication of chronic hepatitis B or C infection.

HCV sustained virological response (SVR): Undetectable HCV RNA in the blood at a defined time point after the end of treatment usually at 12 or 24 weeks (SVR12 or 24)

HCV non-response: Detectable HCV RNA in the blood throughout treatment.

HCV relapse: Undetectable HCV RNA during treatment and/or at end of treatment but subsequent detectable HCV RNA following treatment cessation.

HCV viral breakthrough: Undetectable HCV RNA during treatment followed by detectable HCV RNA despite continued treatment.

HBV treatment failure: May be primary or secondary. Primary antiviral treatment failure may be defined as failure of an antiviral drug to reduce HBV DNA levels by ≥1x log10 IU/mL within 3 months of initiating therapy. Secondary antiviral treatment failure may be defined as a rebound of HBV DNA levels of ≥1 x log10 IU/mL from the nadir in persons with an initial antiviral treatment effect (≥1 x log10 IU/mL decrease in serum HBV DNA).

Serological assays: Assays that detect the presence of either antigens or antibodies typically in serum or plasma but also in capillary/venous whole blood and oral fluid. These include rapid diagnostic tests (RDTs), and laboratory-based immunoassays, *e.g*. enzyme immunoassays (EIAs), chemiluminescence immunoassays (CLIAs), and electrochemiluminescence immunoassays (ECLs).

Rapid diagnostic test (RDT): Immunoassays that detect antibodies or antigens and can give a result in less than 30 minutes. Most RDTs can be performed with capillary whole blood collected by finger-stick sampling.

Enzyme immunoassay (EIA): Laboratory-based serological immunoassays that detect antibodies antigens, or a combination of both.

Nucleic acid testing (NAT): A molecular technology for example, polymerase chain reaction (PCR) or nucleic acid sequence-based amplification (NASBA) that can detect very small quantities of viral nucleic acid (RNA or DNA), either qualitatively or quantitatively.

Multiplex or multi-disease testing: Refers to testing using one specimen in the same test device (or reagent cartridge) that can detect other infections (*e.g.* HIV, syphilis, hepatitis C, hepatitis B).

Clinical/diagnostic sensitivity of a test: The ability of a test to correctly identify those with the infection or disease (i.e. true positives/true positives + false negatives).

Clinical/diagnostic specificity of a test: The ability of a test to correctly identify those without the infection or disease (i.e. true negatives/true negatives + false positives). Sensitivity and specificity are usually expressed as point estimates accompanied by confidence intervals.

Positive predictive value (PPV): The probability that when a person's test result is positive they truly have the infection/disease.

Negative predictive value (NPV): The probability that when a person's test result is negative they truly do not have the infection/disease. Predictive values are influenced by the prevalence of the disease in the population.

Analytical sensitivity/Limit of detection (LoD): The lowest concentration of measurement that can be consistently detected in 95% of specimens tested under routine laboratory conditions. It defines the analytical sensitivity in contrast to the clinical or diagnostic sensitivity.

Testing algorithm: The combination and sequence of specific assays used within hepatitis B and C testing strategies.

Testing approach: In the context of the guidelines mentioned in this book the testing approach describes both "who to test" i.e. different populations, and "where to test" i.e. different settings. Testing approaches include general population testing, focused testing of high-risk groups, "birth-cohort" testing, or of antenatal clinics. These can be delivered through either health-facility or community-based testing.

Testing strategy: A general sequence of assays for a specific testing objective or approach taking into consideration the presumed disease prevalence in the population being tested. A one-assay serological testing strategy involves a single serological assay. A two-assay serological testing strategy involves two different serological assays used sequentially.

Key populations: Groups of people who due to specific high-risk behaviors, are at increased risk for HIV infection irrespective of the epidemic type or local context. This may also apply to HBV and/or HCV infection. Key populations often have legal and social issues related to their behaviors that increase their vulnerability to HIV, HBV, and HCV infection. These guidelines refer to the following groups as key populations men who have sex with men (MSM); people who inject drugs (PWID); people in prisons and other closed settings; sex workers; and transgender people.

Vulnerable populations: Groups of people who are particularly vulnerable to HBV/HCV infection in certain situations or contexts. These guidelines refer to the following groups as vulnerable populations migrant and mobile workers, and indigenous populations.

General population testing: This approach refers to routine testing throughout the entire population without attempting to identify high-risk behaviors or characteristics. It means that all members of the population should have potential access to the testing program.

"Birth cohort" testing: This approach means routine testing among easily identified age or demographic groups (i.e. specific "birth cohorts") known to have a high HCV prevalence due to past generalized exposures that have since been identified and removed.

Antenatal clinic testing: This approach means routine testing of pregnant women especially in settings where there is an intermediate or high seroprevalence to identify women in need of antiviral treatment for their own health and additional interventions to reduce mother-to-child transmission (MTCT).

Community-based testing: Includes using outreach (mobile) approaches in general and key populations; home-based testing (or door-to-door outreach); testing in workplaces places of worship, parks, bars, and other venues; in schools and other educational establishments; as well as through campaigns.

Facility-based testing: Includes testing in primary care clinics inpatient wards, and outpatient clinics, including specialist dedicated clinics such as HIV, STI, and TB clinics, in the district, provincial or regional hospitals and their laboratories, and in private clinical services.

Integration The co-location and sharing of services and resources across different disease areas. In the context of hepatitis B or C infection, this may include the provision of testing, prevention, care, and treatment services alongside other health services, such as HIV, tuberculosis (TB), sexually transmitted infections (STI), antenatal clinic (ANC), contraceptive and other family planning services.

Decentralization: The process of delegating significant authority and resources to lower levels of the health system (provincial regional, district, sub-district, primary health care, and community.

Task-shifting/sharing: The rational redistribution of tasks from "higher-level" cadres of healthcare providers to other cadres such as trained lay providers.

Lay provider: Any person who performs functions related to healthcare delivery and has been trained to deliver services but has received no formal professional or paraprofessional certificate or tertiary education degree.

Linkage to care: A process of actions and activities that support people testing for HBV/HCV to engage with the prevention treatment, and care services as appropriate for their hepatitis B and C status.

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Prof. Imran Shahid earned his bachelor's in Science and Pharmacy from the Islamia University of Bahawalpur, Bahawalpur, Pakistan with distinction. He completed his M.Phil and Doctoral degree in Molecular Biology from the National Center of Excellence in Molecular Biology, University of Punjab, Lahore, Pakistan, while working on anti-mRNA-based treatment strategies against hepatitis C infection. He joined the College of Pharmacy, Umm Al-Qura University, Makkah, Saudi Arabia in 2012 and was involved as a lead scientist in two national projects on hepatitis C pathogenesis and breast cancer treatment, respectively. Currently, he is working as an assistant professor at the Faculty of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia, where his research is focused on broad areas of virus-host networks in HCV disease progression and their therapeutic interventions. He has published many research and review articles in peer-reviewed international journals and is an active member of the editorial board of several international journals.

Qaiser Jabeen

Prof. Qaiser Jabeen graduated in Pharmacy from the Islamia University of Bahawalpur, Bahawalpur, Pakistan in 1995. She started her professional career as a Lecturer in 1996 in the parent institution. She completed her M.Phil. and Ph.D. in Pharmacology from the University of Karachi, Pakistan, while working as an active member of the natural products Pharmacology group at the Aga Khan University, Karachi, Pakistan. She served IUB as an Assistant Professor and was followed by an associate Professor for seven years each. Currently, she is working as a Professor and Chairperson of the Department of Pharmacology, Faculty of Pharmacy, the Islamia University of Bahawalpur. She has attended many national and international conferences and seminars and is working as an active member of several committees at the university level. She has a good number of publications of international repute and book chapters to her credit. She is also an active editorial member of various national and international journals.