Angelos Hatzakis *Editor*

Hepatitis C: Epidemiology, Prevention and Elimination

Volume 1

Springer

Hepatitis C: Epidemiology, Prevention and Elimination

Angelos Hatzakis
Editor

Hepatitis C: Epidemiology, Prevention and Elimination

Volume 1

Springer

Editor
Angelos Hatzakis
Department of Hygiene, Epidemiology and
Medical Statistics
National and Kapodistrian University of
Athens Medical School
Athens, Greece

ISBN 978-3-030-64648-6 ISBN 978-3-030-64649-3 (eBook)
https://doi.org/10.1007/978-3-030-64649-3

This Springer imprint is published by the registered company Springer Nature Switzerland AG.
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Contents

HCV Virology

1

Eve-Isabelle Pécheur, Fabien Zoulim, and Birke Bartosch

1.1 Experimental Systems to Study HCV Life Cycle

In parallel to these key discoveries, adequate and improved in vitro and in vivo models were developed. Sophisticated cell culture systems were designed to facilitate HCV propagation and establish long-term culture systems. The human hepatoma cell line Huh7 was first used as culture model for HCV infection, but without much success. Huh7 cells can produce interferons (IFN), potent physiological antiviral molecules. Several lines of evidence indicated that HCV replication intermediates activate IFN regulatory factor 3 (IRF-3), and induces IFN production in these cells [1] via the IFN-inducible cellular DExD/H box RNA helicase RIG-I, which upon binding to HCV RNA triggers IRF-3 via its caspase activation and recruitment domain homologue (CARD). The selection of an Huh7 cell clone exhibiting a dominant negative mutation in the CARD homology domain of RIG-I led to the discovery of the Huh7.5 clone, which is highly permissive for HCV replication. Huh7.5 cells produce high levels of infectious virions and maintain persistent infection over several passages [2, 3]. However, the general impact of RIG-I/IRF-3 signaling on permissiveness to HCV replication in these cells has been a matter of debate, and the issue is still unresolved. While some authors regard RIG-I as a cytosolic pathogen sensor/receptor, the inactivation of which leads to increased

E.-I. Pécheur
UMR5286, CNRS, INSERM U1052, Lyon Cancer Research Center, Lyon, France

Department of Immunology and Virology, University of Lyon, Lyon, France
e-mail: eve-isabelle.pecheur@inserm.fr

F. Zoulim · B. Bartosch (✉)
UMR5286, CNRS, INSERM U1052, Lyon Cancer Research Center, Lyon, France

Department of Immunology and Virology, University of Lyon, Lyon, France

DevWeCan Laboratories of Excellence Network (Labex), Lyon, France
e-mail: Fabien.Zoulim@inserm.fr; Birke.Bartosch@inserm.fr

A. Hatzakis (ed.), *Hepatitis C: Epidemiology, Prevention and Elimination*,
https://doi.org/10.1007/978-3-030-64649-3_1

permissiveness [1, 4], others have shown that (a) inactivation of IRF-3 in Huh7 cells does not modify HCV replication efficiency, nor increases permissiveness, and (b) restoration of functional RIG-I signaling in Huh7.5 cells does not alter HCV replication [5]. These discrepancies might be due to the use of varying technical approaches and the use of different HCV replicons and corroborate with the observation that RIG-I signaling is only poorly activated by single-stranded (ss) RNAs and in particular by HCV ssRNA. Thus, upon HCV entry into its host cell, HCV RNA, released from the nucleocapsid, can be quickly translated with only minimal activation of RIG-I. This leads to the synthesis of viral proteins. Importantly, the viral protease NS3/4A is able to degrade Cardif, an adaptor of RIG-I indispensable for subsequent IRF-3 activation. Only at later stages of replication will double-stranded (ds) RNA intermediates appear that could efficiently activate the RIG-I signaling pathway [5]. Studies aimed at defining the molecular determinants of the interaction between dsRNA and RIG-I point to the presence of a 5′-triphosphate and a minimal length of dsRNAs, together with the restriction of RIG-I ATPase activity as critical for RIG-I signaling activation [6, 7].

The use of Huh7.5 cells led to the discovery of two of the four essential receptors required for HCV entry, claudin-1 [8] and occludin [9, 10], and led to the identification of other receptor molecules and entry factors, such as the Niemann-Pick C1L1 molecule [11], and the receptor of the epidermal growth factor [12], and the heparan sulfate proteoglycan syndecan-1 [13, 14]. Human primary hepatocytes are the most physiologically relevant cells to perform in vitro infections, closely reproducing the in vivo situation [15], but their survival in culture is limited and their capacity to sustain productive infection is low. To overcome these limitations, human embryonic pluripotent stem cell-derived hepatocytes (hESC) were isolated, differentiated into hepatocyte-like cells (HLC), and successfully infected with HCV, able to complete a full replication cycle [16]. Similar successful infection experiments could be mounted with human-induced pluripotent stem cells (hiPSC) derived from patients [17].

HCV in vivo studies were long hampered by the lack of (small) animal models able to sustain HCV infection and develop humanlike disease symptoms. Chimpanzees were instrumental to the discovery and identification of HCV as the etiologic agent for non-A non-B viral hepatitis [18]. However, the natural course of infection in chimpanzees differs from that in humans, since very few develop chronic HCV infection and no fibrosis and only one hepatocellular carcinoma (HCC) case have been observed [19]. Moreover, availability, cost, and ethical concerns limit the use of these primates for HCV research. Support for invasive research on chimpanzees was recently discontinued in the USA. Small animal (rodent) models were therefore developed. The first fully permissive murine model that supported long-term HCV infection was produced by intrasplenic injection of primary human hepatocytes into immunodeficient mice with diseased liver, such as Alb-uPA/SCID (urokinase-type plasminogen activator transgenic severe combined immunodeficiency) and FRG (fumarylacetoacetate hydrolase-recombination activating gene 2-interleukin-2 receptor, common γ-chain knockout) [20–22]. These mice with human chimeric liver secrete human serum albumin levels

similar to those observed in humans [23], and display a humanlike blood lipoprotein profile [24] that renders them highly susceptible for infection with human hepatotropic pathogens such as HCV and the hepatitis B virus, thus enabling the study of HCV biology, the evaluation of different antiviral strategies, and the occurrence of antiviral resistance [20, 25]. However, these mice are immunodeficient, and active research was conducted to develop a fully immunocompetent mouse model of HCV infection with hepato-pathological manifestations [26].

1.2 Structural Organization of Viral Particles

HCV isolated from the serum of infected patients, chimpanzees, or mice with humanized livers can be fractionated into roughly three populations: a very compact and heavy fraction, a fraction of intermediate density, and a population of very light particles floating at the surface of density gradients [27]. HCVcc display a similar biophysical behavior [28]. Observed by transmission electron microscopy (TEM), the three populations appear respectively as (a) 35–40 nm nonenveloped particles; (b) 70 nm spherical particles, comprising an electron-dense core and a lipid envelope identified as a bilayer, and considered as canonical viral particles; and (c) particles with the aspect of serum lipoproteins, called lipo-viro-particles (LVPs; see below and Fig. 1.1).

All particles have in common the presence of a nucleocapsid composed of the single-stranded viral RNA compacted by the capsid protein called core. Particle populations of intermediate and light densities are enveloped fractions harboring the

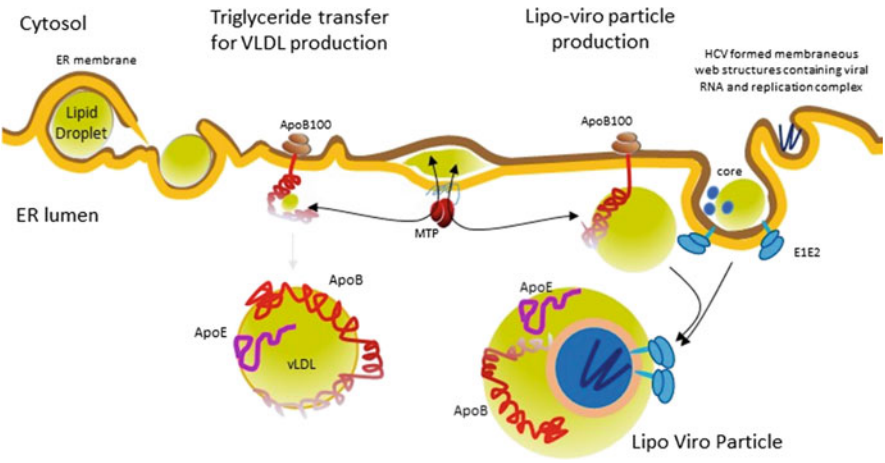


Fig. 1.1 A simplified scheme of lipoviral particle assembly: assembly occurs at the ER at the interface between the HCV-induced membranous web containing the HCV replication complex and lipid droplets where core and NS5A are localized. LVP production depends on cellular VLDL assembly and secretion, which is mediated by lipidation of nascent ApoB via mitochondrial triglyceride transfer protein (MTP). See text for further details

glycoproteins E1 and E2 embedded in the lipid membrane surrounding the nucleocapsid. Core, E1, and E2 constitute the structural proteins of HCV. At the virion surface, E1 and E2 form a heterodimer assembled by covalent interactions and stabilized by disulfide bridges [29].

1.2.1 Nonenveloped Nucleocapsids

A high-density fraction of viral particles isolated from HCV-infected patients was found composed of the HCV core, associated with HCV RNA. Electron microscopy revealed particles of heterogeneous size, with the predominant population 38–43 nm in diameter [30]. Similar structures were found in HCV produced in cell cultures (HCVcc) [31], with a mean diameter of 44 nm. However, these fractions displayed only low infectivity, so they are unlikely to be infectious.

1.2.2 Canonical Particle Structure

This intermediate-density pool of viral particles constitutes about 50% of the total particle population of HCV grown in cell cultures (clone JFH-1). This pool has been characterized at the ultrastructural level by cryo-TEM: analysis revealed particles of an average diameter of 60–64 nm, composed of a 5–6-nm-thick electron-dense bilayer, surrounding the nucleocapsid viewed as the densest zone [31]. These particles are characterized by a high E2/core protein ratio and contain HCV RNA. As a matter of fact, this fraction of in vitro-produced particles had the highest infectivity. However, particles produced in vitro from another clone (Jc1), or in vivo in mice with humanized livers, displayed a lower but more homogeneous density profile, and a higher specific infectivity than JFH-1 HCVcc, and were found enriched with apolipoprotein E (apoE; see below) [23, 32]. The lipid envelope of JFH-1 HCVcc was highly enriched in cholesterol as compared to cellular membranes and displayed a high cholesterol-to-phospholipid content [33]. Together with sphingomyelin, virion-associated cholesterol played a key role in HCV infectivity. The envelope of Jc1 HCVcc showed similar cholesterol contents as those measured for cellular membranes, but was found enriched in sphingomyelin [32], a lipid composition comparable to that of low-density lipoproteins (LDL) of human serum.

1.2.3 LVP Structure

A hallmark of HCV particles is their association with host cell lipids and lipoproteins, mainly very-low-density lipoproteins (VLDL) and LDL, which are known to impact virion assembly, infectivity, and structure [27, 28, 34, 35] (see Fig. 1.1). In patient, sera virions display a very low buoyant density and are associated with lipoproteins. Based on these findings, HCV virions have been called

lipo-viro particles (LVPs) [27]. Biochemical as well as electron microscopy-based approaches have confirmed LVPs as hybrid particles composed of viral components and cellular lipoprotein components including in particular apolipoproteins E, B100/48, CI, CII, and CIII [36]. The dependence of HCV assembly on the cellular lipid metabolism is probably responsible for the strong morphological heterogeneity and dynamic structural changes of LVPs that have been observed in response to changes in dietary triglycerides [34, 37]. In serum, HCV components have been identified in various forms: LVPs, composed of an electron-dense center encapsidating the HCV RNA genome surrounded by a detergent-sensitive lipid coat. Interestingly, nucleocapsids are frequently detected at the periphery of the LVP and not in the center. LVPs also contain, besides the envelope glycoprotein heterodimer, E1/E2, ApoE, and B100 at the surface. Subviral lipoprotein-like particles, which contained E1/E2 and ApoE and B but lacked the nucleocapsid and HCV genome, were also detected in patient sera [38]. Interestingly, lipoprotein-like particles represent a predominant form of HCV in the blood, suggesting that they may play a major role in HCV immune responses and evasion. Indeed, reversible association of ApoE with LVPs in the serum has been shown to protect viruses from neutralization and modify their interactions with heparan sulfate proteoglycans (HSPG) [39]. However, the exact roles of LVPs in persistence as well as transmission remain to be determined.

1.3 The Viral Proteins

The HCV particles contain a positive polarity RNA genome with 5′ and 3′ untranslated regions (UTR) and a long open reading frame encoding a polyprotein precursor of about 3000 amino acids. UTRs constitute highly conserved, cis-acting RNA elements regulating viral genome translation and replication. Translation of the polyprotein is initiated by ribosome binding to an internal ribosome entry site (IRES), which spans most of the 5'-UTR and the first 24–40 nucleotides of the core coding region. This results in the production of a single precursor polyprotein, which is processed by cellular and viral proteases into ten structural and nonstructural proteins (core, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B). Core protein and the envelope glycoproteins (E1 and E2) make up the structural components of the virion. Nonstructural proteins (from NS3 to NS5B) assemble into a membranous-web-associated HCV RNA replicase complex that catalyzes the amplification of the viral RNA genome.

1.3.1 Core

It is a multifunctional protein. One of its main functions is to form the viral capsid with the genomic RNA, thereby contributing to particle assembly. The monomeric, mature form of HCV core is a 21 kDa protein with lipid and RNA-binding activities [40]. The three-dimensional structure of core is still unknown, but biophysical

studies revealed that the protein forms dimers. The monomers are organized in two domains, D1 and D2. A large part of the D1 domain is intrinsically disordered. This feature might contribute to core RNA chaperoning functions, critical for the structural remodeling and packaging of the RNA genome into the viral particle [41]. As other intrinsically unstructured proteins, core is expected to adopt different conformations depending on the presence of specific cellular partners, involved in gene transcription, lipid metabolism, apoptosis, and cell signaling. Through its mainly α-helical D2 domain, core associates to the membranes of the endoplasmic reticulum (ER) and lipid droplets (LDs) [42] (Fig. 1.1), where efficient viral replication, assembly, and infectious particle production occur. Comprehensive mutagenesis studies revealed residues critical for HCV infectivity, but not for viral RNA replication, evidencing the key role of core in HCV particle formation [43].

Core recruits nonstructural proteins, HCV RNA, and the replication complex to LD-associated membranes [44]. In particular, core recruits NS5A to LDs through direct protein/protein interactions, at regions enriched in HCV glycoproteins, E1 and E2. This spatial vicinity between structural proteins involved in particle formation and nonstructural proteins of the replication complex closely links particle assembly to virus replication. It was therefore suggested that LDs can serve as platforms for virion formation [44]. Core also interacts with host cell factors that play a role in the viral assembly process. Diacylglycerol acetyltransferase-1 (DGAT1), a key enzyme of LD biogenesis, binds core and targets it to LDs; the inhibition of this trafficking impairs HCV particle production [45]. DGAT1 was also shown to facilitate the binding of NS5A to core and guide both proteins onto the surface of LDs, aiding infection [46]. Nucleoporin-98 is a structural component of the nuclear pore complex, which interacts with HCV core and relocalizes to LDs [36]; it is a key factor involved in late steps of viral particle biogenesis, whose functions are most likely hijacked by HCV to efficiently transport its proteins from various compartments. The tail-interacting protein of 47 kDa (TIP47) also contributes to the targeting of NS5A to core-enriched HCV assembly sites on the surface of LDs, by direct TIP47/NS5A interactions [47].

1.3.2 E1 and E2

HCV virions harbor at their surface two transmembrane type I proteins, E1 and E2, in the form of a covalent heterodimer stabilized by disulfide bridges [29]. The transmembrane domains of E1 and E2, located at their C-termini, are involved in heterodimerization and have ER retention sequences [48]. E1 and E2 are heavily glycosylated, with respectively 5 and 11 conserved N-linked glycans. This N-linked glycosylation plays key roles in (a) HCV assembly by regulating the folding of E1 and E2 and the formation of the E1/E2 heterodimer; (b) HCV entry into target cells by modulating the affinity of viral particles for cell surface receptors, in particular CD81 (see below and Fig. 1.2); and (c) HCV susceptibility to neutralizing antibodies by contributing to the evasion of HCV from the humoral immune response [49]. Both proteins are involved in the steps of viral entry and fusion, unlike other

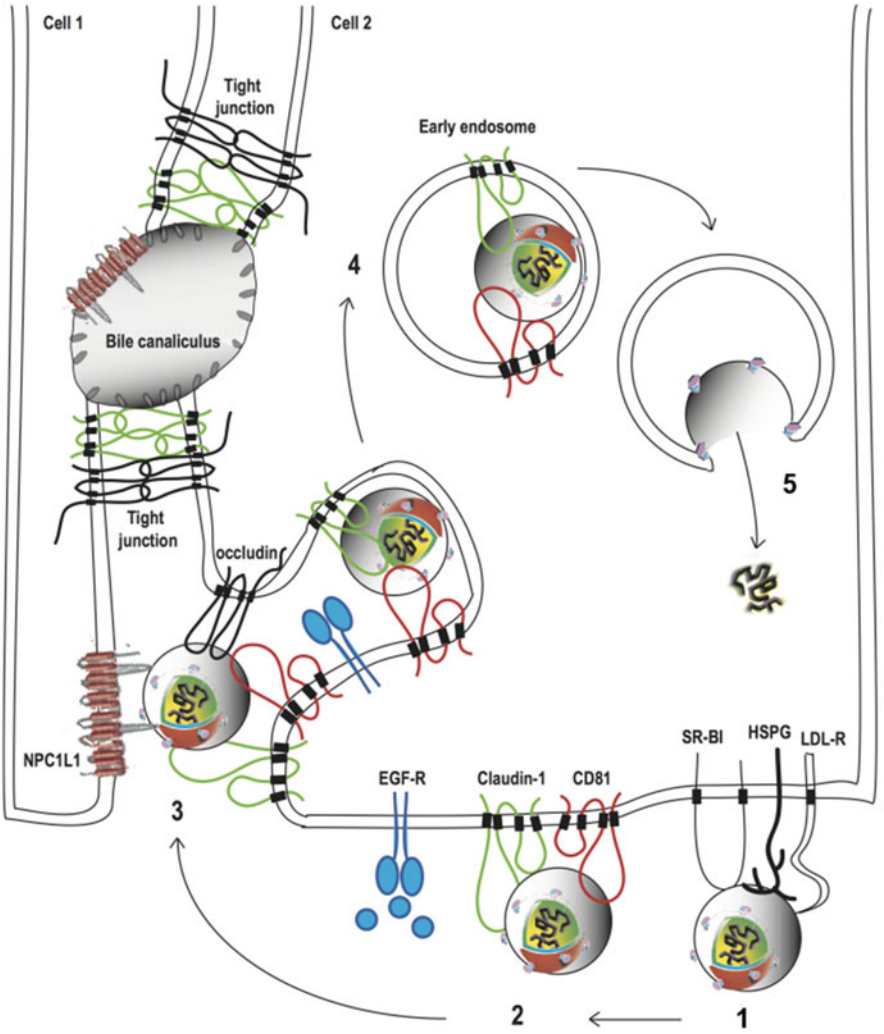


Fig. 1.2 HCV entry into hepatocytes is a multistep process. HCV arriving from the blood and associated with serum lipoproteins attaches to heparan sulfate proteoglycans (HSPG) and recognizes the receptor for low-density lipoproteins (LDL-R) and the scavenger receptor SR-BI at the basolateral surface of hepatocytes (*1*). This initial binding and recognition step is followed by the recognition of the tetraspanin CD81 (*2*), of the receptor for epidermal growth factor EGF-R, of components of the tight junction claudin-1 and occludin, and of the cholesterol absorption molecule NPC1L1, at the apical biliary pole (*3*). The viral particle is then internalized together with CD81 and claudin-1 via clathrin-mediated endocytosis in an early endosome (*4*), where fusion takes place between viral envelope and endosomal membrane (*5*), mediated by HCV envelope glycoproteins E1 and E2 (in blue and pink, respectively). This leads to the release of HCV nucleocapsid into the cytosol to initiate RNA translation and viral replication

members of the *Flaviviridae* family such as alphaviruses or flaviviruses where E1 or E trimers, respectively, are sufficient. Due to their high glycan content, HCV E1 and E2 long resisted attempts to produce and purify them at high yields and to determine their 3D-structure.

1. E1 is a 192aa protein. In the absence of high-resolution structure, it was modeled in silico as a truncated class II fusion protein, mainly composed of β-sheet folds [50]. The 3D-structure of its N-terminal domain has recently been solved by X-ray crystallography [51] and revealed an unusual and totally unexpected architecture for a protein (domain) presumably involved in processes of viral entry and fusion. It forms a covalent homodimer, and its closest structural homologue is a human phosphatidylcholine transfer protein in complex with a phospholipid. Although it is still unclear if this structure corresponds to the structure on the virion, a post-attachment structure, or a post-fusion fold, this opens novel perspectives in terms of lipid binding capacity of the viral particles. Indeed, the N-terminal domain of HCV E1 has been suggested to be responsible for the binding to ApoB and ApoE, which are components of LVPs. This same domain was also suggested to facilitate virus entry through either low-density lipoprotein receptor or heparan sulfate [52, 53]. At the surface of HCV virions, E1 forms a trimer, most likely assembled through interactions in the transmembrane domains [54]. The formation of this trimer depends on the presence of E2, and this trimeric form is essential for virus infectivity. This configuration was recently confirmed by computational analyses, supporting a trimeric arrangement of E1/E2 further assembled into a pentamer, with 12 pentamers comprising a single HCV virion [55].

E2 is a 363aa glycoprotein, composed of three hypervariable regions called HVR1, HVR2, and the intergenotypic variable region (igVR), a stem region, and the transmembrane domain. E2 is the receptor-binding protein, shown to interact with the tetraspanin CD81 and the scavenger receptor BI (SRB1), two HCV co-receptors (see Fig. 1.2). The HVR1 region plays an instrumental role in HCV entry into hepatocytes, by contributing to E2-SRBI interactions [56, 57]. E2 is also the major target of neutralizing antibodies, and the first neutralizing epitopes described on HCV E2 were found within HVR1 [58]. Anti-HVR1 antibodies neutralize HCV by interfering with E2-SRBI interactions [59, 60]. HCV can continuously escape the host neutralizing response by mutations, resulting in loss of recognition of HCV envelope glycoproteins by antibodies; the genetic evolution of the envelope is therefore shaped by neutralizing antibody pressure, in particular in the HVR1 region [61]. HVR1 conceals the CD81 binding site on E2, thereby decreasing exposure of conserved epitopes [62]. HVR1 may thus act as an immunological decoy, diverting the immune system.

Since E2 is twice the size of E1, it was hypothesized to be the fusion protein of HCV, by analogy to the E protein of the dengue [63, 64] and tick-borne encephalitis flaviviruses [65], or to the E1 protein of the Semliki Forest alphavirus

[66]. Therefore, it has long been considered as a class II fusion protein rich in β-strands, containing putative fusion peptides [67, 68] (chapter on "Viral Entry/Fusion" for more details). A truncated version of E2 was recently generated and complexed with a neutralizing antibody, which was amenable to X-ray diffraction. As for E1, the high-resolution structure of E2 revealed an unexpected fold. Indeed, most of the protein residues are either in loops or intrinsically disordered [69, 70]. Only one β-sandwich region is shared with presumed structural homologues from flaviviruses and alphaviruses. This truncated HCV E2 protein, which maximally measures ~50 Å, does not adopt the extended class II fusion protein fold (100–120 Å) [71].

Taken together, the recent structural data for HCV E1 and E2 do not support the prediction that they are class II fusion proteins. Moreover, one predicted putative fusion peptide of E2 is contained within the hydrophobic core, ruling out membrane fusion activity. The E2 ectodomain also does not undergo major rearrangements upon exposure to low pH [69]. This strongly suggests that E2 is not a fusion protein. The N-terminus of E1 lacks any structural resemblance to other viral fusion proteins. Therefore, HCV membrane fusion might be a completely different mechanism from that described for other *Flaviviridae* and related viruses such as alphaviruses.

In the ER of infected hepatocytes, HCV E1/E2 glycoproteins, ApoB, and ApoE colocalize and form a ternary physical complex [72]. This complex then readily associates with intracellular infectious viral particles and is found conserved in the secreted infectious viral particles. This strongly suggests that the complex formed by HCV E1/E2, ApoB, and ApoE may initiate LVP morphogenesis.

1.3.3 P7

P7 is a member of the family of viral viroporins, such as Vpu of the human immunodeficiency virus (HIV-1), E5 of the human papillomavirus 16 (HPV16), and the M2 protein of the influenza A virus. It is classified as a viroporin because it oligomerizes to form hydrophilic pore/ion channels. It is a 63aa membrane-spanning protein, essential for efficient virus particle assembly and release [73]. The structure of p7 is still a matter of debate mainly due to its sensitivity to the solvent or lipid environment generally used for purification. The oligomerization state of p7 has been described as a hexamer [74], and recent computer simulations yield hexameric and heptameric channels [75]. P7 has nonselective pore properties and, like several other viroporins, displays a low ion selectivity, with minor preferences for cations vs. anions. It has proton conductance properties and may act to prevent acidification in otherwise acidic intracellular compartments. This action is required for productive HCV infection [76]. Therefore, p7 might be regarded as a modulator of the pH-mediated maturation process of HCV particles as a first stage and as a key factor to render secreted virions pH-resistant as a second stage.

Regardless of its ion channel activity, p7 interacts with the protein NS2. This serves to recruit the envelope glycoproteins E1 and E2 to the ER membrane sites of HCV assembly and regulates core localization to these sites [77]. Direct interactions

between p7, core, and HCV glycoproteins have been reported in infectious cell systems [78]. P7 is also necessary for the final steps of capsid assembly and for capsid envelopment, linking capsid assembly to membrane envelopment of nascent RNA-containing core protein multimers [73]. As the HCV glycoproteins interact with ApoB and ApoE [72], these data suggest that p7 may influence apolipoprotein incorporation into HCV particles or particle maturation, thus contributing to virus production and infectivity.

1.3.4 NS2

The 217aa long NS2 protein is membrane-associated via three putative transmembrane segments with a perinuclear ER localization in its N-terminus [79, 80]. The C-terminal protease domain (aa 94–217) resides on the cytoplasmic face of the ER membrane [80], and its activity is strongly enhanced by the N-terminal domain of NS3 [81, 82]. NS2/NS3 catalyze the cleavage at the NS2/NS3 site [83, 84] which frees fully functional NS3 protein. The NS2 protease domain is highly conserved and requires dimerization for the formation of a composite active site with a catalytic triad analogous to those of cysteine proteases [85]. NS2 expressed alone has been shown to exhibit only low-level intrinsic protease activity, and as mentioned, its protease activity is stimulated by the NS3 serine protease domain (residues 1–180) [86]. Mutational analysis revealed a hydrophobic NS3 surface patch that mediates NS2 protease stimulation as well as NS5A hyperphosphorylation and viral RNA replication [83, 85, 87]. The finding that NS2 dimerization is required for proteolysis suggests that certain concentrations of the protein have to be accumulated before NS2–3 processing, and thus initiation of RNA replication can occur. Within this time window, the virus may generate sufficient amounts of NS3/4A to antagonize the activation of the interferon pathway, which is necessary to allow the onset of viral replication (see below) [88].

NS2 protein, independently of its proteolytic activity, is required for viral assembly [81, 82, 89–91]. Several laboratories have shown that full-length NS2 is required for particle production [92]. Construction of chimeric HCV genomes derived from different genotypes revealed that interactions of the N-terminal transmembrane segment of NS2 with upstream structural proteins as well as interactions between the C-terminus of NS2 with downstream nonstructural proteins are likely required for virion assembly [93]. Moreover, NS2 was shown to co-localize with several other HCV proteins, and in particular with E2 and NS5A, raising the question whether NS2 may induce membrane alterations and act as bridge to mediate interactions between structural virion components and the viral RNA replication complex that are required for assembly, encapsidation, and release. NS2 has in addition been reported to interact with various cellular proteins resulting in interference with apoptosis, inhibition of cell proliferation, inflammatory processes, hepatic lipid metabolism, and DNA repair [94–99], but most of these data remain to be validated in physiologically relevant replication systems.

1.3.5 NS3/4A

NS3 is a 70 kDa protein with a serine protease domain located in the N-terminus and NTPase/RNA helicase activity in the C-terminal two thirds of the protein [100]. It forms a chymotrypsin-like fold with two beta-barrel subdomains. NS3 associates non-covalently with the 54aa long cofactor NS4A. The central residues 21–32 of NS4A form a beta-strand that is integrated into the N-terminal beta-barrel of NS3, while the N-terminus forms a transmembrane helix that mediates membrane association of the NS3/4A complex. The C-terminus of NS4A (aa 40–54) forms an alpha helix that interacts with replicase components and is required for viral RNA replication and assembly [101]. The substrate specificity of NS3/4A remains ill-defined, but is thought to be determined by a rather common consensus cleavage sequence as well as positioning of the NS3/4A complex in respect to the membrane [102]. Indeed, membrane association of NS3/4A is determined in a sequential manner by the amphipathic alpha helix in the N-terminus (aa 12–23) of NS3 and the N-terminal alpha helix (aa 1–21) of NS4A. Importantly, NS3/4A is not only located on ER membranes but also at mitochondria and mitochondria-associated membranes [103, 104], where the NS3/4A complex cleaves and thus inactivates the innate immune sensor MAVS [105]. An additional NS3/4A substrate important for viral immune evasion is importin beta1, which is required for IRF3 and NF-kB signaling [106]. The NTPase/RNA helicase activity of NS3 couples ATP hydrolysis to double-stranded RNA unwinding and is required for viral RNA replication and assembly [102].

1.3.6 NS4B

NS4B is the least characterized of the HCV proteins due to its hydrophobicity, strong membrane association, and lack of well-defined enzymatic functions. NS4B is an oligomeric membrane protein of 261 aa located on the cytoplasmic side of the ER membrane [107]. The N-terminus of NS4B is thought to be at least partially translocated into the ER lumen [108] and consists of two the amphipathic alpha helices AH1 (aa 3–35) and AH2 (aa 42–66). Oligomerization of the AH2 helix is thought to mediate transition of the membrane bilayer [107, 109]. Furthermore, site directed mutagenesis of AH1 and AH2 has implied these helixes in virion production [110] and assembly of the viral replication complex [111]. The central part of NS4B (aa 191–261) comprises four predicted transmembrane domains, the C-terminus consists of two strongly conserved amphipathic alpha helices (aa 201–213; aa 229–253). The central transmembrane domains of both, N- and C-terminal helices, play important roles in membrane association. NS4B induces the formation of particular membranous vesicles in ER- or ER-derived membranes, within which viral replication takes place [112, 113]. Among the factors that drive the vesicle formation process are the oligomerization of NS4B and certain host cell factors known to interact directly with NS4B [114, 115]. The induction of membranous vesicles by NS4B may be due to its capacity to induce membrane curvature which in

turn maintains nonstructural proteins and viral RNA inside newly formed replication vesicles [116–118]. Indeed, NS4B interacts with other viral nonstructural proteins and binds viral RNA [119]. In addition, NS4B displays NTPase activity and is required for viral assembly [120]. While NS4B is considered the main driver of membranous vesicle formation, other viral proteins participate. Recent data show that also the sole expression of NS3/4A, NS5A, and even NS5B can trigger vesicle formation [121]. Interestingly, membrane vesicles triggered by the expression of individual viral proteins are different from those observed upon expression of the entire NS3–5B cassette, which results in formation of double-membrane vesicles [121].

1.3.7 NS5A

NS5A is a 447-amino-acid-long zinc-binding and proline-rich hydrophilic phosphoprotein with pleiotropic roles in the HCV life cycle. NS5A alters NS5B polymerase activity in vitro, is required for viral RNA replication and assembly, and modulates cell signaling pathways, interferon responses, and apoptosis. NS5A is composed of an N-terminal amphipathic helix of 30 aa that mediates membrane association [122] followed by three structural domains separated by two low-complexity sequences [123]. Domain I (aa 28–213) is known to bind RNA and lipid droplets and is essential for viral RNA replication [118, 124–126]. It has been crystallized as a dimer in two different configurations [127, 128] pointing to distinct functional conformations and/or multimerization of NS5A. Domains II (aa 250–342) and III (aa 356–447) are naturally unstructured and known to interact with a number of different viral and cellular proteins [129, 130]. Roles of these domains in RNA replication and viral assembly have recently been described [131–133]. Phosphorylation has been implicated as regulatory switch that modulates several functions of NS5A depending on the ratio of phosphorylated versus hyperphosphorylated forms [134, 135]. While basal phosphorylation has been observed at central and C-terminal residues, highly conserved serine residues located between aa 214 and 249 have been shown to be hyperphosphorylated [133, 134, 136].

The IFN sensitivity-determining region (ISDR) at the C-terminus of NS5A has been reported to contain strong trans-activating capacities, suggesting that NS5A likely functions as a transcriptional activator [137]. In addition, NS5A has been shown to interact with a number of cellular factors [138, 139]. In particular, the activation of phosphatidylinositol-4-kinase alpha (PI4KA) by NS5A is critical for the creation of the viral replication compartment. PI4P, the product of PI4KA activity, recruits, e.g., cellular lipid transfer proteins to this replication compartment, such as the cholesterol transporter oxysterol-binding protein [140]. In addition, PI4KA modulates the phosphorylation status of NS5A [141]. NS5A is also known to interact with other cellular factors and pathways that either impact the HCV life cycle as well as associated pathogenic processes. Among these are, e.g., pro-proliferative targets such as p53/p21 [142], the cell cycle kinases cdc2 and cdk2 [143], and Grb2 [144]. Interactions between NS5A and apoptosis regulating

proteins such as Bax [145], members of the Src family of tyrosine kinases [137], and modulation of epidermal growth factor receptor (EGFR) trafficking and signaling [146, 147], possibly via interactions with cellular SH3-domain-containing, are also likely to have proviral as well as pro-oncogenic effects. Finally, NS5A has been shown to co-localize with core protein on lipid droplets and to interact with different lipoproteins [148]. In particular, NS5A may cause inhibition of ApoB100 secretion [149].

1.3.8 NS5B

During HCV replication, the positive-strand RNA genome is used as the template for an RNA-dependent RNA polymerase (RdRp), which synthesizes a complementary negative-strand RNA and subsequently genomic positive-strand RNA. Both of these steps are catalyzed by the RdRp NS5B [150], which has rapidly emerged as a major target for antiviral intervention (see below, chapter "Direct-Acting Antivirals and Their Mode of Action"). This protein contains motifs shared by all viral RdRps, and has the classical fingers, palm, and thumb subdomains, based on the similarity of the enzyme structure with the shape of a right hand. The palm domain is the most highly conserved domain across different polymerases and the location of the active site. In contrast, the thumb domain is the most variable. Fingers and thumb domains vary significantly in both size and secondary structure depending on the specific requirements for replication in a given virus [151]. A special feature of the HCV RdRp is that extensive interactions between the fingers and thumb subdomains result in a completely enclosed active site. HCV NS5B functions as an oligomer, which was reported to be important for cooperative RNA synthesis activity [152]. It is associated with the ER membrane through its C-terminus (tail-anchored protein) [153], and membrane targeting occurs by a posttranslational mechanism. NS5B has been recently identified as a cofactor of HCV assembly through a genetic association with the p7 viroporin contributing to virion-specific infectivity [154].

1.4 Life Cycle

1.4.1 Entry

Viral entry is defined as the stages by which a virus penetrates into and delivers its genetic material into the host cell. This does not necessarily lead to productive infection, and frequently the process of entry is being studied regardless of the capacity of the virus to replicate or not in infected target cells. General features of viral entry comprise the attachment of viral particles to cell surface molecules, the recognition of specific receptors at this surface, followed by internalization of virions in intracellular compartments by mechanisms that depend or not on pH. This leads to viral fusion, which triggers the release of the viral genetic material into the cytoplasm of the target cell. Studies of HCV entry have long been hampered by the

absence of relevant virus/cell culture models. Major advances were made with the advent of HCVpp as a surrogate model of HCV particles [155]. HCVpp consist of a retroviral nucleocapsid and harbor the HCV glycoproteins E1 and E2 at their surface in the form of a noncovalent E1/E2 heterodimer [156, 157]. Many key observations on HCV entry obtained with the HCVpp model have retrospectively been validated using infectious cell culture-grown HCV virus, HCVcc.

The process of HCV entry is initiated by physical interactions between structural elements of the virions (E1/E2 heterodimer, apolipoproteins) and host cell surface molecules (see Fig. 1.2). The heparan sulfate proteoglycan (HSPG) syndecan-1 is an element of the extracellular matrix, reported to bring about viral attachment to the surface of hepatocytes [13, 14] through the involvement of virion-associated apoE [52]. The HSPGs syndecan-2 and syndecan-4 were also reported to play a role in viral attachment, but to a lesser extent than syndecan-1 [158]. Syndecan-1 forms a complex with the tetraspanin CD81 [13], a key HCV receptor [56, 159], thereby linking the process of attachment to that of receptor recognition. Through its numerous glycosaminoglycan moieties and their high level of sulfation specific to liver cells, as well as through its engagement in a complex with CD81, syndecan-1 may contribute to HCV hepatotropism.

Initially described as an HCV receptor [57, 160], the lipoprotein-binding molecule scavenger receptor BI (SRBI) has also been described as an attachment factor, involving the apoE moiety of HCVcc particles for initial interactions [161]. HCV entry mediated by SRBI also relies on the lipid transfer function of this molecule and on interactions between the hypervariable region 1 (HVR1) region of E2 and SRBI. This receptor is also a key entry factor for virions produced in mice with humanized livers [23]. However, SRBI engagement in the entry of such particles seems to solely rely on its lipid transfer function and to be independent of E2 [23].

Since HCV associates with serum lipoproteins, the involvement of the receptor for low-density lipoproteins (LDL-R) in HCV entry has been suggested [162, 163]. However, engagement of this receptor seems to lead to nonproductive infection as virions end up in degradation compartments such as lysosomes [164]. Its implication in HCV entry deserves further in vivo investigations.

CD81 is a member of the tetraspanin web, which coordinates extracellular and intracellular processes, in particular through its link with the cytoskeleton [165]. Tetraspanins have four transmembrane passages and two extracellular loops. HCV virions recognize a patch of amino acids in CD81, present in the large extracellular loop [159, 166], through specific residues of E2 contained in the recently published three-dimensional structures [69, 70]. The HVR1 region of E2 is thought to conceal CD81-binding sites [62], suggesting that structural rearrangements are necessary for E2-CD81 interactions. As described above, syndecan-1 and CD81 form a complex, involved in HCV internalization in intracellular acidic compartments [13].

Claudin-1 and occludin are integral components of the tight junctions that delineate the basolateral from the apical membranes of hepatocytes. These molecules were reported to play a key role in HCV entry [8–10, 167], and to compose the minimal quartet of receptors required for efficient HCV entry, together with SRBI

and CD81. Claudin-1 forms a complex with CD81, instrumental to viral particle internalization [168]. The component Sec24C of the coat protein complex II (COPII) machinery of intracellular protein transport was found crucial for proper claudin-1 transport to and exposure at the hepatocyte cell surface, which identifies this molecule as a key cofactor of HCV entry [169]. After infection, the expression of claudin-1 and occludin is downregulated [9]; this downregulation serves to prevent secondary infection of already infected hepatocytes, a phenomenon known as exclusion of superinfection [170]. Similar observations have been described for syndecan-1 [13].

The receptor of the epidermal growth factor (EGFR) and ephrin receptor A2 (EphA2) have been identified as host cofactors of HCV entry [12]. Entry involves the receptor tyrosine kinase (RTK) activity of both receptors, as shown in in vitro and in vivo models of HCV infection. Engagement of these molecules in HCV entry is achieved by regulating the association of CD81 and claudin-1, thereby activating the GTPase HRas [171]. Indeed, the current view is that HCV binding to CD81 induces RTK phosphorylation, thereby leading to HRas activation. HRas may then promote lateral diffusion of CD81 within the plasma membrane and its stable clustering with Claudin-1, prior to subsequent virus internalization.

In the search for the involvement of lipid receptor molecules in HCV/LVP entry, the cholesterol transporter Niemann Pick C1-like1 (NPC1L1) and the receptor for very-low-density lipoproteins (VLDLR) were identified as entry factors. NPC1L1 is a 13-transmembrane domain protein of the apical membrane of hepatocytes. It is involved in cellular cholesterol absorption and contributes to cholesterol homeostasis. The implication of this molecule in HCV entry expands the view that virion-associated cholesterol plays a key role in viral entry [11]. However, the exact nature of HCV/NPC1L1 interactions remains unclear. NPC1L1 expression is downregulated during HCV infection, which might contribute to the exclusion of superinfection, as suggested for claudin-1, occludin, and syndecan-1.

Most recently, the VLDLR was identified as a new molecule of the (complex) pathway of HCV access to hepatocytes [172]. Surprisingly, HCV infection using VLDLR does not require any of the above-described HCV receptors; therefore VLDLR-mediated HCV infection may be different from previously reported entry mechanisms. The interaction between HCV and VLDLR requires E2 and the apoE part of the LVP.

Our current model of HCV entry implies that multiple interactions take place at the surface of hepatocytes for subsequent efficient entry. Virion binding to attachment factors may lead to structural rearrangements of protein components of the viral particle, with the exposure of previously masked regions in the E1/E2 heterodimer. SRBI is the first receptor molecule to be recognized in the cascade of HCV entry events [173]. This triggers the binding to CD81, induces EGFR phosphorylation and activation of the GTPase HRas, and drives the lateral diffusion of CD81 molecules in the membrane plane and their clustering at claudin-1-enriched sites, where virions also likely interact with occludin. Virions are then internalized through a clathrin-dependent endocytosis process involving the small GTPase Rab5 [174, 175], together with syndecan-1 complexed with CD81 [13]. At this stage,

NPC1L1 might alter the lipid composition of the viral envelope, to facilitate subsequent viral fusion.

While the predominant site of viral replication is hepatocytes, some lines of evidence suggest that HCV is also lymphotropic. HCV has been shown to interact with and use B cells as vehicle to optimize its transmission into the liver; however, no active replication in B cells was observed [176]. With the recent identification of the immune cell-specific, co-stimulatory receptor B7.2 as a co-receptor of lymphotropic HCV strains, strong evidence has now emerged that suggests that HCV may actively replicate in B cells [177].

HCV infection can be achieved by two modes: (a) a cell-free transmission, where virions come from the blood circulation and infect naïve hepatocytes, or transit outside an infected cell to reach adjacent targets, and (b) via cell-to-cell transmission, where the virus propagates between adjacent hepatocytes without being exposed to the extracellular medium. This latter mode of viral transmission is particularly efficient to escape the immune system and presumably facilitates the establishment of chronic infection. The cell-to-cell transmission route was found dependent on SRBI, claudin-1, and occludin [178, 179] and on the E1/E2 heterodimer of HCV [180]. The NPC1L1 molecule also plays a role in this mode of transmission, in a manner involving its cholesterol transporter properties, and maybe in conjunction with the tight junction components claudin-1 and occludin, located in the vicinity of NPC1L1 at the hepatocyte apical membrane [181]. ApoE incorporated onto the viral particle was found to play a key role in HCV cell-to-cell spread, thereby linking viral assembly to viral propagation [182]. In another study, apoE was found dispensable to this mode of HCV transmission [181]; these conflicting data might evolve from the use of different cellular models. The involvement of CD81 in HCV cell-cell propagation is still a matter of debate: initial findings suggested that CD81 is dispensable [180]. However, cumulating pieces of evidence now suggest that CD81 plays at least a partial role in cell-to-cell transmission [13, 178, 183].

The process of viral fusion is the stage where the viral envelope merges with cellular membranes through the action of viral fusion proteins. This leads to the release of the viral genetic material in the cytosol. After their internalization in intracellular compartments through clathrin-dependent endocytosis, HCV virions are exposed to low pH in the endosomes, where conformational rearrangements of their envelope glycoproteins are likely to occur. At present and in spite of the recent publication of the 3D-structures of both E1 and E2, it is impossible to determine which protein triggers membrane fusion. Experimental evidence using HCVpp and HCVcc in combination with artificial membranes (liposomes) or cellular membranes show that HCV-induced fusion of liposomes is pH-dependent, over a large range of pH of 6 to 4 which would correspond to early to late endosomal compartments in cellulo [68, 174, 184]. CD81 and claudin-1 are involved in HCV fusion in endosomal compartments positive for the small GTPase Rab5 [174]. CD81 was found to prime HCV for low pH-dependent fusion, likely through conformational rearrangements in the E1/E2 heterodimer [185]. Fusion is also strongly dependent upon cholesterol and sphingomyelin present in the target membranes [68, 184] and in the viral envelope [11].

1.4.2 Replication

Soon after fusion and release of HCV positive-strand RNA into the cytosol, replication begins in a peculiar compartment derived from the ER, called membranous web [112]. At the ultrastructural level, this web is composed of single- and double-membrane vesicles and contains markers of rough ER, early and late endosomes, transport COP vesicles, mitochondria, and lipid droplets (LDs) [121]. Double-membrane vesicles are induced by NS5A, while NS4B induces single-membrane vesicles. Within this membranous compartment, active replication and assembly of de novo synthesized viral machineries take place (see "Assembly" chapter). NS3 to NS5B are minimal viral protein components required for RNA replication. NS5B is the RNA-dependent RNA polymerase and constitutes the catalytic core of the replication machinery. Purified NS5B is not very efficient at replication, suggesting cellular cofactors are required in addition. NS5B can be phosphorylated by the interacting cellular protein kinase C-related kinase 2 (PRK2), and depletion of PRK2 inhibits HCV replication, suggesting that the PKR2-mediated phosphorylation is important for function [186]. Interaction with the cellular chaperonin TRiC/CCT is also important for NS5B function [187], but the underlying mechanism is not elucidated.

NS4B drives the scaffold formation required for HCV RNA replication, which takes place in membranous vesicles. The viral replication complex contains besides NS4B also NS3/4A, NS5A, and NS5B. Physical interactions between these viral proteins lead to formation of the membrane-associated multiprotein complex that coordinates RNA replication [188–191]. NS5B is the key enzyme of RNA synthesis and uses the positive incoming RNA genome to generate a first negative-strand genome, which in turn serves as template for positive-strand RNA synthesis. These steps produce double-stranded RNA intermediates. The newly replicated positive-strand RNA can then be reused for replication or translation or be packaged into virions. The 3′end of the HCV negative-strand is an excellent template for de novo initiation, whereas the 3′end of the positive-strand hardly gives rise to terminal de novo initiation, probably because it is buried within a stable stem structure [192, 193]. Indeed, auxiliary factors are required for and control initiation of negative-strand synthesis by NS5B [194]. After binding, NS5B synthesizes a dinucleotide primer [195], and a conformational change in the polymerase structure then allows egress of the template-primer duplex [196, 197]. RNA synthesis then proceeds at 100–400 nts/min and is highly error-prone, resulting in the high genetic variability of HCV isolates [198]. The 3′ non-translated region (NTR) is essential for initiation and regulation of negative-strand RNA synthesis [192, 199]. It is composed of a variable region, which forms two stem-loop structures, a polyU/UC tract and a conserved 98-nucleotide-long X-tail, which also comprises three stem-loop structures at the end of the viral genome [200]. Additional stem loops have been identified within the NS5B coding region that are implied in the formation of a kissing-loop interaction with the second stem loop in the X-tail. This interaction, which forms a pseudoknot structure at the 3′ end of the genome, is essential for RNA replication [201–203]. NS3, NS5A, as well as NS5B have all been shown to bind to

the polyU [125, 199, 204–206]. However, the distinct functional role of the polyU/UC region in viral RNA synthesis has not been clarified yet. The 5′NTR has a dual function in genome replication. It contains an IRES sequence and thus drives translation of the positive RNA strand. The 5′NTR and a complementary stretch at the 3′end of the negative-strand RNA have been shown to adopt particular secondary structures. In particular, two adjacent miR-122 seed sequences located in the 5′NTR point to a primordial role of this miR in the proper formation of RNA secondary structures. The two adjacent sites as well as the intermittent, strongly conserved 8 nt spacer are all important for HCV replication and translation [207, 208].

Different lines of evidence suggest roles of NS3/4A and NS5A in RNA replication, but the underlying mechanisms have not been fully elucidated. NS3 helicase activity is thought to resolve the stem-loop structures in the 3′NTR to facilitate de novo replication initiation by NS5B. Structure determination of NS5A suggests that NS5A forms dimers that bind RNA [124, 128]. Thus, NS5A may play a role in RNA transport. In addition, NS5A may interact with cellular proteins that impact viral replication. Indeed, NS5A- and NS5B-stimulated PI4KA activity is required for membranous vesicle formation and HCV RNA replication [209]. In addition, HCV replication depends on altered membrane structure, for which the expression of genes that regulate the hepatic lipid metabolism is necessary [210–212]. In addition, posttranslational modifications, e.g., phosphorylation, geranlygeranylation, or palmitoylation of cellular and viral factors, are required for viral replication [213]. Geranylgeranylated FBL2, for example, interacts with NS5A and this is critical for HCV RNA replication [214]. Oligomerization of NS4B depends on palmitoylation of C-terminal cysteine residues [107]. HCV core [215–217] and NS5A [148, 218] are located on lipid droplets, which are frequently found in the vicinity of membranous vesicles induced by HCV. LDs, or rather core protein on the surface of LDs, are thought to coordinate replication with assembly, possibly via a direct interaction with NS5A [44, 131, 133, 219]. In addition, LDs may also play an important role in RNA replication [220].

Among the long list of host factors involved in RNA synthesis are, e.g., the human VAMP-associated protein A (hVAP-A) and its isoform hVAP-B [221, 222], which play a role in cellular vesicle transport and are thought to impact HCV-induced membrane rearrangements (reviewed in [223]). hVAP-A, e.g., binds to hypophosphorylated NS5A and is thought to regulate viral replicase activity in an NS5A phosphorylation-dependent manner [224]. Prolactin regulatory element-binding protein, which regulates anterograde ER-Golgi transport, interacts with NS4B, relocalizes to the viral replication complex, and is also required for membranous vesicle formation [115]. Within the family of the peptidyl-prolyl cis/trans isomerases, cyclophilin B was initially found to interact with NS5B and to regulate template binding of the polymerase [225]. More recent data point however to a role of cyclophilin A (CyPA) in HCV replication [226, 227] by modulating conformation of NS5A [226] and biogenesis of the membranous web [228]. Binding of the liver-specific, highly abundant microRNA miR-122 to two seed sequences in the 5′NTR probably prevents degradation by RNases, stabilizes the viral genome [229],

stimulates translation of the viral RNA, and prevents activation of innate immune responses [230].

As mentioned above, NS5B has an intrinsically high error rate estimated at 2.5×10^{-5} mutations per nucleotide per genome replication [231]. In addition, selective pressure exerted by antiviral immune responses drives evolution of the genetic diversity of HCV. HCV is today classified into seven genotypes, characterized by differences in over 30–33% of nucleotide positions. In addition, HCV genotypes 1–6 have been classified into subtypes that differ in at least 15% of nucleotide positions [232]. HCV genetic variability is not evenly distributed across the viral genome. The 5′-NTR region and viral capsid are the most conserved regions of the genome, while the most variable region codes for the envelope glycoproteins E1 and E2. In particular, the sequences encoding HVR1 and HVR2 of E2 display the least sequence homology between different isolates [233, 234].

1.4.3 Assembly

Fractionation studies have shown that membranes composing the membranous web contain NS5B, viral RNA, phosphatidylinositol 4-kinase III-alpha (PI4KIIIα), ApoE, core protein, and infectious HCV [235, 236]. At early stages of assembly, core associates with lipid droplets (LDs) followed by the recruitment of the replication complex [44], in an NS2-dependent manner [77]. LDs therefore play a central role in the coordination of viral RNA synthesis and virion assembly, by physically associating replication and assembly sites. Recently, the heterogeneous nuclear ribonucleoprotein K (HNRNPK) was found recruited close to LDs, where it co-localized with core and with the HCV plus-strand RNA [237]. HNRNPK might limit the availability of viral RNA for incorporation into virions, thereby determining efficiency of HCV particle production. The recruitment of NS5A to either LDs or assembly sites in the ER (through interaction with core protein) is also required for proper viral particle assembly [238]. The correct distribution of HCV NS5A to replication and assembly sites is under the regulation of the ATPase p97/VCP (valosin-containing protein) [239], a cytosolic enzyme involved in particular in the fusion of Golgi membranes [240]. NS5A recruitment to LDs is also promoted by the tail-interacting protein 47 (TIP47), through direct protein interactions [47]. The interactions between NS5A and core are stabilized by the host molecular chaperone ApoJ, thus facilitating infectious HCV particle production [241].

HCV virions are LVPs and HCV production depends on VLDL assembly and secretion [242]. Thus, the roles of VLDL-associated proteins such as apoB, apoE, apoA1, apoC1, and microsomal triglyceride transfer protein (MTP) in the formation of infectious HCV particles have been addressed. ApoB and MTP were found dispensable for infectious particle production [182]. NS5A and the E1/E2 heterodimer interact with apoE [72, 243–245]. ApoE is a key component of HCV virions, required for virion infectivity and production [246]. While budding, the viral particle interacts with nascent or immature lipoproteins, which indicates that the lipoprotein secretion machinery is involved in the formation of HCV viral particle

[247]. Mechanistically, the current evidence suggests that (a) viral capsids bud into the ER lumen, followed by the incorporation of large amounts of cholesteryl esters and triglycerides into these nascent HCV particles; (b) apoB and exchangeable apolipoproteins (A1, C1, E) bind to these lipid-rich particles as if they were lipoproteins; and (c) these HCV enveloped particles fuse with lipoproteins to generate LVPs [248].

HCV assembly is modulated by several host factors, of which most are related to lipid metabolism and LDs. Nucleoporin-98, a structural element of the nuclear pore complex, has been recently described as a key factor of HCV morphogenesis, whose nuclear functions are diverted by HCV for the transport of viral proteins to guide assembly [36]. Diacylglycerol acyltransferase 1 (DGAT1) performs the last step in triacylglycerol synthesis pathway and is involved in LD biogenesis, together with DGAT2. DGAT1 plays a direct role in the recruitment of core and NS5A to LDs, through direct interactions with core [45]. The small GTPase Rab18 is a LD-associated protein that binds NS5A, thereby physically linking LDs to ER membranes. Its GTP-bound (active) form is prominent over the GDP-bound (inactive) one for this activity [249]. Rab32, another small GTPase of the Rab family, was recently described to play a role in core recruitment to ER-derived membranes, likely at virion assembly sites [250]. Unlike Rab18, Rab32 displayed this activity under its GDP-bound form. The cytosolic group IVA phospholipase A2 gamma (PLA2G4A), involved in the release of arachidonic acid, was shown to affect the amount of core on LDs and the efficiency of core envelopment [251]. The phosphatidylinositol 3,5-bisphosphate 5-phosphatase FIG 4 is implicated in HCV morphogenesis and infectivity through its capacity to modulate the amounts of cholesteryl esters [252]. ABHD5 (α/β hydrolase domain-containing protein 5) was recently identified as a host factor promoting both virus assembly and release [253]. ABHD5 associates with LDs and triggers their hydrolysis, and HCV usurps ABHD5 lipase cofactor function. HCV also recruits the lipid-binding protein annexin A3 to LDs to achieve proper morphogenesis [254]. Cell-death-inducing DFFA-like effector B (CIDEB), a regulator of the VLDL pathway associated with LDs, was shown to be required for HCV assembly [255]. A kinase involved in the innate pathway, IκB kinase-α (IKK-α), has been described as a crucial host factor for HCV assembly: HCV, through its 3′ untranslated region, interacts with DEAD box polypeptide 3, X-linked (DDX3X) to activate IKK-α, which translocates to the nucleus and induces a transcriptional program involving sterol regulatory element-binding proteins (SREBPs) [256]. Consequently, this innate pathway induces lipogenic genes and enhances core-associated LD formation to facilitate viral assembly.

1.4.4 Maturation/Release

According to the current model of HCV assembly, E1-/E2-enriched ER membranes encompass luminal lipid droplets, into which hydrophobic nucleocapsids become enclosed. Similar to the production of VLDL, where lipid-poor VLDL precursors fuse with luminal LDs, these immature virion particles are thought to fuse with

luminal lipid droplets or VLDL precursors to form properly lipidated "virus-like particles" (LVP). Among the VLDL assembly machinery, in particular ApoE is thought to play a primordial role in this intracellular lipidation by mediating fusion between nascent VLDL and viral precursors [182, 243, 246, 254, 257, 258]; however ApoE does not affect formation of HCV nucleocapsids and their envelopment per se [245]. Indeed, particles can efficiently be produced in sh-apoE cells, but have increased density and low specific infectivity [245]. While association with ApoE during the assembly process renders particles infectious [257, 259], only particles containing sufficient ApoE amounts are secreted. Mechanistically, the role of ApoE in HCV lipidation and secretion remains unclear, even though direct interactions between ApoE and the glycoproteins E1/E2 have been implied [182, 245]. Recently annexin A3 (ANXA3), a protein recruited to lipid-rich fractions in HCV-infected cells, has been shown to be essential for interactions between ApoE and the E2 glycoprotein. Curiously, ANXA3 silencing affected trafficking, but not lipidation of HCV virions [254]. Reconstitution of HCV particle assembly in non-hepatic cell lines has shown that besides ApoE members of the ApoA and ApoC apolipoprotein families can also sustain HCV particle lipidation and secretion, and the required conserved structural domains in these apolipoproteins have been identified as amphipathic alpha-helical repeats [182, 260].

HCV virions then bud from the ER and traffic to the Golgi with COPII-vesicles [257, 261]. It has also been shown that localization of ARF GTPases such as CYTH3 and ARF3 to the trans Golgi network (TGN), where they interact with coat proteins to regulate vesicle budding and sorting, is necessary for HCV secretion. Release of HCV from the TGN requires furthermore PRKD1. Secretion is then thought to occur via a clathrin-dependent transendosomal secretory route and depends on components of the endosomal-sorting complex required for transport pathway [257, 262–265]. Both viral glycoproteins, E1 and E2, possess high mannose and complex N-linked glycans and essential disulfide bonds that are thought to be rearranged in the Golgi during secretion [29, 54]. To prevent premature fusion of the glycoprotein complex, nascent virions may be protected from acidification and consequent conformational changes via the ion channel activity of p7 [76]. Interestingly, autophagy has been shown to impact HCV assembly and secretion via exosomes [266, 267]. Interactions of HCV particles with the TIP47-GTP-Rab9 complex are thought to protect the nascent virus from autophagosomal-mediated degradation during release [266]. Finally, secreted ApoE can modify infectivity of already released, circulating HCV virions, and thus protect virions from antibody-mediated neutralization and enhance particle interactions with cellular HSPGs and thus potentially facilitate cell entry [39].

1.5 Direct-Acting Antivirals (DAA) and Their Mode of Action

Thanks to the knowledge of the molecular biology of HCV and the development of cell culture assays, direct-acting antivirals (DAAs) were discovered and developed. It was known from early clinical and experimental observations that infected

hepatocytes could be cured by IFN administration [268, 269]. With the discovery of novel DAAs, it was shown that the cure of infected cells could occur in an IFN-independent manner. The first generation of DAAs was mainly directed against HCV genotype 1 [270]. It was rapidly observed that a combination of these drugs with IFN or other antiviral agents would be necessary to prevent the emergence of drug resistance. The second generation of drugs was developed to be pan-genotypic and to confer a high genetic barrier to resistance when administered in combination. Pan-genotypic direct-acting antivirals have been developed for several viral proteins. The predominantly used DAA combinations that are currently in clinical use target the NS3/4A protease, NS5A, or the NS5B RNA-dependent RNA polymerase. Due to extremely high efficacies of these DAA regimens of close to 100% independently of genotype and fibrosis stage, the concomitant use of interferon has been stopped over the last few years, and current therapies are predominantly based on DAAs only. We will focus predominantly on the currently used classes of DAAs and only briefly mention DAAs targeting additional viral proteins.

In respect to the HCV proteases NS2 and NS3, only compounds that target the NS3 protease have reached the clinic. Based on the crystal structure of NS3 in complex with candidate compounds, the two structurally related HCV protease inhibitors telaprevir and boceprevir were developed and the first protease inhibitors (PIs) to be approved for clinical use in 2011. They are linear peptidomimetic structures that react reversibly with the catalytic serine of NS3 to form a covalent bond [271, 272]. To avoid the emergence of drug resistance, these antiviral agents had to be combined with IFN and ribavirin. The major limitations of these first-generation macrocyclic PIs were strong side effects, inconvenient dosing regimens, narrow genotype specificity, and low genetic barrier to resistance. Resistance-conferring mutations that emerged during therapy were predominant at positions R155, 156, and 168 and affected binding to the inhibitor; in contrast changes in binding to natural NS3 substrates were minimal [273]. The second wave of first-generation macrocyclic PIs, such as asunaprevir and simeprevir, had similar limitations [274]. In contrast, the second-generation pan-genotypic PIs, derived from the peptide substrate of NS3/4A protease, were modified with various tailor-made amino acids in order to achieve high sustained virologic response (SVR) against HCV [275]. Among these second-generation PIs, glecaprevir showed potent activity against all genotypes with equal and even superior results compared to grazoprevir and paritaprevir in replicon assays but was found clinically effective only in combination therapies. Grazoprevir retains activity against the common R155K mutant viruses, as it does not interact with this residue [276]. In an interferon-free context, grazoprevir in combination with ribavirin (RBV) resulted in 90% sustained viral response (SVR) in patients with undetectable HCV RNA 4 weeks into treatment; however, in patients with detectable RNA at week 4, the SVR rate was only 58% [277]. Resistance to treatment with the acylsulfonamide paritaprevir is uncommon, because it targets the protease-binding site, but requires surveillance.

Two classes of clinically relevant NS5B inhibitors have been described: non-nucleotide inhibitors (NNIs) and nucleotide inhibitors (NIs) [278]. They act at

distinct stages of RNA synthesis. Initiation of RNA synthesis by NS5B is slow, does not need a primer, and is accompanied by frequent dissociation events. Formation of the first phosphodiester bond then stabilizes the complex and triggers the elongation phase. NS5B is known to be present in functionally distinct complexes in these two phases. Crystal structures of NS5B have indeed shown that NS5B undergoes drastic conformational changes to accommodate the newly synthesized RNA strand in the elongation phase [279]. NNI are chemically diverse and generally interfere with the conformational dynamics at the transition from the initiation to the elongation phase. NNI binding pockets are allosteric sites, which mediate noncompetitive mechanisms of action and are located in distinct regions in the thumb or palm regions. Examples for thumb-targeting NNIs are benzimidazole, indole scaffolds such as beclabuvir, or thiophene-based inhibitors. In contrast to inhibitors targeting the thumb region, palm site inhibitors such as benzothiadiazine scaffolds are thought to inhibit initiation of RNA synthesis through interference with nucleotide incorporation. Importantly, the antiviral activity of NNIs is very limited to predominantly genotype 1. Dasabuvir, the first approved NNI, has a low genetic barrier to resistance and was therefore part of complex combinations with NS5A and NS3A inhibitors. In contrast to NNIs, NIs compete with the incoming nucleoside triphosphate for binding and incorporation, but modifications of their sugar moiety mediate the inhibitory, chain-terminating effect. Because NIs are administered as prodrugs, they need to be processed into the active triphosphate form that can then access the nucleotide-binding site of NS5B [280]. The only currently approved NI drug sofosbuvir is a phosphoramidate prodrug that is intracellularly hydrolyzed and further modified into its active triphosphate form, a uridine analogue containing a 2′-fluoro-*C*-methyl motif. This motif is thought to cause chain termination via a steric conflict with the incoming nucleotide substrate [279]. Sofosbuvir has pan-genotypic antiviral activity, and although it was originally approved with PEG-IFN and ribavirin, it is highly effective when combined with other DAA classes, either as dual or triple DAA combinations in an interferon-free context. Generally, sofosbuvir is well tolerated with a good safety profile, but reports of hepatotoxicity have emerged in the patients with decompensated cirrhosis [281]. Other NIs are currently at early stages of clinical development.

Unlike NS3 and NS5B, NS5A does not possess any enzymatic activities. Furthermore, the limited structural information that is currently available for NS5A is based on non-phosphorylated proteins. Hence, the development of DAAs for NS5A has been difficult. Inhibitors were identified in cell-based replicon assays that screened for the emergence of resistance-conferring mutations in the NS5A coding region. NS5A inhibitors show exceptional potencies, with EC_{50} values in the picomolar range. Daclatasvir, the first NS5A inhibitor, and the related compounds ledipasvir and ombitasvir are in clinical use [282]. Resistance-conferring mutations, e.g., L31 and Y93 in genotype 1, have been used to identify the putative-binding site of the inhibitors. Indeed, several models for complexes of NS5A bound to daclatasvir or related compounds have been proposed. Recent quantitative-binding approaches suggest that daclatasvir competes with RNA such that a dynamic equilibrium occurs between NS5A dimers that bind either RNA or daclatasvir

[283]. The high potency of these NS5A inhibitors has also been shown to be due to the inhibition of DMV formation. NS5A inhibitors are among the most potent DAAs developed to date, display an excellent safety and pan-genotypic efficacy profile, and are therefore present in most combination treatments that are currently being applied in the clinic [284]. However, resistance mutations have been observed that persist on the long term [285]. The NS5A inhibitor pibrentasvir, currently being evaluated as pan-genotypic regimen in combination with the second-generation protease inhibitor glecaprevir, shows a high genetic barrier to resistance and potency against common NS3 and NS5A polymorphisms and seems efficient even in the setting of compensated cirrhosis [286].

Further clinical results based on these DAAs will be treated in subsequent chapters.

1.6 Host-Targeting Agents and Their Mechanisms of Action

Given the high amount of host factors involved in HCV life cycle, we will only focus on those factors suggested to represent potential targets for host-targeting agents during distinct steps of HCV infection. Basically, each step of HCV life cycle is amenable to antiviral therapy, with several molecules already licensed for their anti-HCV potential. Moreover, several molecules targeting different steps could be combined to achieve a better efficiency and to lower the doses of each individual molecule, thereby increasing the therapeutic index of the medication. Finally, HTAs could be successfully associated with DAAs to gain in antiviral potency.

Entry inhibitors have long been designed to block the virus before it causes damages to its host and to protect naïve cells from infection. A vast panel of molecules, from fully synthetic ones to extracts of natural compounds, have been proposed and assayed. However, only a little number could be reasonably proposed for antiviral strategies, since most of these molecules raise safety concerns and/or display insufficient purity to identify the compound endowed with antiviral activity. In the following, we will therefore only focus on molecules currently under in vivo investigations. Monoclonal antibodies targeting cellular proteins/receptors have long been proposed as antiviral strategies; however, most of these proteins are essential to many cellular functions, so their targeting cannot be applied in vivo for safety reasons. Nevertheless, successful attempts have led to the development of monoclonal antibodies directed toward CD81 [287], claudin-1 [288], and SRBI [289] that showed potent antiviral activity in mice with humanized livers. The compound ITX-5061 inhibits SRBI-mediated lipid transfer from high-density lipoproteins (HDL), but not the binding of HDL to SRBI [290]; this promotes HDL levels, and since SRBI activity is targeted, HCV entry is inhibited. ITX-5061 is currently under phase 1 clinical investigation, in chronically infected patients receiving a liver transplantation (NCT01292824 and NCT01560468) [291]. The involvement of the EGFR and of NPC1L1 in HCV entry allowed the use against hepatitis C of specific inhibitors of these receptor molecules, already licensed with other therapeutic indications. In this context, erlotinib, an anticancer agent used in lung cancer and

inhibiting the tyrosine kinase activity of EGFR, was assessed in a clinical trial of chronic hepatitis C (NCT01835938), with no results posted yet. The inhibitor of the NPC1L1 cholesterol transporter ezetimibe is an already approved molecule against hypercholesterolemia; it showed potent anti-HCV activity both in vitro and in vivo in mice with human livers [11] and has now entered two clinical trials (NCT02126137 and NCT02971033). Silymarin is a natural extract from milk thistle (*Silybum marianum* sp.), shown to display anti-HCV activity in clinical settings [292]; however, its efficacy was questioned since it comprised several molecules. Silibinin is the main active compound of silymarin that showed potent antiviral activity, in vitro [174, 293] and in patients [294]. It acts by slowing the clathrin-dependent endocytosis of HCV particles and by altering the recruitment of Rab5 to endosomes, which leads viral particles to degradation compartments [174]. It has also an inhibitory effect on HCV replication [295] and assembly [293]. A recent clinical trial showed favorable treatment outcomes with silibinin in difficult-to-treat patients coinfected with HCV and HIV [296]. The antimalarial chloroquine is a chemical analogue of ferroquine, shown to exhibit an inhibitory activity on HCV infection, at the stages of cell-to-cell transmission and fusion [297]. Chloroquine is an affordable molecule for emerging countries, and has recently entered a phase 4 clinical trial in Iran (NCT02058173), with favorable but modest antiviral outcomes [298].

Inhibitors of host cell factors involved in HCV replication have been designed, and some used with success in clinical settings. Miravirsen, an inhibitor of micro-RNA 122, a liver-specific micro-RNA that binds to conserved sites in the 5′-UTR of the viral RNA and is necessary for viral replication and translation [208, 299], has proven its efficacy in reducing viral loads in chronic hepatitis C patients in phase 1–2 clinical trials [300, 301]. Miravirsen may act by two complementary mechanisms: it might hybridize to mature miR-122, thereby blocking its interaction with HCV RNA, and bind to the stem-loop structure of miR-122 precursors, leading to impaired processing of these molecules. Importantly, miR-122 may play an important role in the suppression of pro-fibrogenic actions of stellate cells; indeed its downregulation is associated with fibrogenesis [302, 303]. Cyclosporins are inhibitors of specific signal transduction pathways that lead to T-lymphocyte activation. These immunosuppressive agents bind with high affinity to the cytoplasmic receptors cyclophilins. Several drugs devoid of immunosuppressive properties were designed and displayed in vitro anti-HCV activity [304]. Alisporivir has been successfully used in clinical settings (NCT02094443, NCT01215643, NCT02753699), with a pan-genotypic anti-HCV activity and a high genetic barrier to resistance; in combination with ribavirin, it displayed a favorable safety profile and a good antiviral efficiency [274]. As already mentioned, cyclophilin inhibitors block HCV replication by preventing the formation of double-membrane vesicles, thereby reorganizing the ER-derived membranous web where viral replication occurs and by preventing the formation of complexes between cyclophilin A and NS5A, thereby inhibiting HCV replication [304].

The list of assembly inhibitors in the clinical process is currently very limited. A most promising host target protein is DGAT-1, shown to play a key role in HCV

morphogenesis and secretion (see above). However, the specific inhibitor LCQ908 that exhibited antiviral potency in vitro proved disappointing in vivo [305], and the corresponding clinical trial was terminated (NCT01387958).

The only host-targeting agents extensively used in the clinic is ribavirin. Ribavirin was part of the standard of care treatment in combination with IFN. Although it did not have a direct antiviral effect, it enhanced the antiviral activity of IFNα. It was maintained in the triple combination regimen with the first protease inhibitors and IFN. With the development of more potent DAA, ribavirin was still used in the IFN-free regimen in combination with other DAAs in difficult-to-treat patients to increase the rate of sustained virologic response. Ribavirin was shown to modify the epigenetic environment of interferon-stimulated genes and thus to restore IFN responsiveness [306]. More recently, with the development of even more potent DAAs, the role of ribavirin in the treatment of CHC patients has vanished.

1.7 Conclusion

The last two decades of research allowed to understand the molecular biology of HCV replication, characterize the crystal structure of most viral proteins, and develop experimental models to study HCV infection. This knowledge assisted the drug discovery efforts that led to the development of potent antiviral drugs. The clinical development of these agents led to a medical revolution as it is now possible to nearly cure all patients with IFN-free regimens.

References

1. Sumpter R Jr, Loo YM, Foy E, Li K, Yoneyama M, Fujita T, Lemon SM, Gale M Jr. Regulating intracellular antiviral defense and permissiveness to hepatitis C virus RNA replication through a cellular RNA helicase, RIG-I. J Virol. 2005;79:2689–99.
2. Blight KJ, McKeating JA, Rice CM. Highly permissive cell lines for subgenomic and genomic hepatitis C virus RNA replication. J Virol. 2002;76:13001–14.
3. Zhong J, Gastaminza P, Cheng G, Kapadia S, Kato T, Burton DR, Wieland SF, Uprichard SL, Wakita T, Chisari FV. Robust hepatitis C virus infection in vitro. Proc Natl Acad Sci U S A. 2005;102:9294–9.
4. Saito T, Hirai R, Loo YM, Owen D, Johnson CL, Sinha SC, Akira S, Fujita T, Gale M Jr. Regulation of innate antiviral defenses through a shared repressor domain in RIG-I and LGP2. Proc Natl Acad Sci U S A. 2007;104:582–7.
5. Binder M, Kochs G, Bartenschlager R, Lohmann V. Hepatitis C virus escape from the interferon regulatory factor 3 pathway by a passive and active evasion strategy. Hepatology. 2007;46:1365–74.
6. Anchisi S, Guerra J, Garcin D. RIG-I ATPASE activity and discrimination of self-RNA versus non-self-RNA. MBio. 2015;6:e02349.
7. Binder M, Eberle F, Seitz S, Mucke N, Huber CM, Kiani N, Kaderali L, Lohmann V, Dalpke A, Bartenschlager R. Molecular mechanism of signal perception and integration by the innate immune sensor retinoic acid-inducible gene-I (RIG-I). J Biol Chem. 2011;286:27278–87.

8. Evans MJ, Von Hahn T, Tscherne DM, Syder AJ, Panis M, Wolk B, Hatziioannou T, McKeating JA, Bieniasz PD, Rice CM. Claudin-1 is a hepatitis C virus co-receptor required for a late step in entry. Nature. 2007;446:801–5.
9. Liu S, Yang W, Shen L, Turner JR, Coyne CB, Wang T. Tight junction proteins claudin-1 and occludin control hepatitis C virus entry and are downregulated during infection to prevent superinfection. J Virol. 2009;83:2011–4.
10. Ploss A, Evans MJ, Gaysinskaya VA, Panis M, You H, De Jong YP, Rice CM. Human occludin is a hepatitis C virus entry factor required for infection of mouse cells. Nature. 2009;457:882–6.
11. Sainz B Jr, Barretto N, Martin DN, Hiraga N, Imamura M, Hussain S, Marsh KA, Yu X, Chayama K, Alrefai WA, Uprichard SL. Identification of the Niemann-Pick C1-like 1 cholesterol absorption receptor as a new hepatitis C virus entry factor. Nat Med. 2012;18:281–5.
12. Lupberger J, Zeisel MB, Xiao F, Thumann C, Fofana I, Zona L, Davis C, Mee CJ, Turek M, Gorke S, Royer C, Fischer B, Zahid MN, Lavillette D, Fresquet J, Cosset FL, Rothenberg SM, Pietschmann T, Patel AH, Pessaux P, Doffoel M, Raffelsberger W, Poch O, McKeating JA, Brino L, Baumert TF. EGFR and EphA2 are host factors for hepatitis C virus entry and possible targets for antiviral therapy. Nat Med. 2011;17:589–95.
13. Grigorov B, Reungoat E, Gentil Dit Maurin A, Varbanov M, Blaising J, Michelet M, Manuel R, Parent R, Bartosch B, Zoulim F, Ruggiero F, Pecheur EI. Hepatitis C virus infection propagates through interactions between Syndecan-1 and CD81 and impacts the hepatocyte glycocalyx. Cell Microbiol. 2016;19:5.
14. Shi Q, Jiang J, Luo G. Syndecan-1 serves as the major receptor for attachment of hepatitis C virus to the surfaces of hepatocytes. J Virol. 2013;87:6866–75.
15. Gondeau C, Briolotti P, Razafy F, Duret C, Rubbo PA, Helle F, Reme T, Ripault MP, Ducos J, Fabre JM, Ramos J, Pecheur EI, Larrey D, Maurel P, Daujat-Chavanieu M. In vitro infection of primary human hepatocytes by HCV-positive sera: insights on a highly relevant model. Gut. 2014;63:1490–500.
16. Roelandt P, Obeid S, Paeshuyse J, Vanhove J, Van Lommel A, Nahmias Y, Nevens F, Neyts J, Verfaillie CM. Human pluripotent stem cell-derived hepatocytes support complete replication of hepatitis C virus. J Hepatol. 2012;57:246–51.
17. Carpentier A, Tesfaye A, Chu V, Nimgaonkar I, Zhang F, Lee SB, Thorgeirsson SS, Feinstone SM, Liang TJ. Engrafted human stem cell-derived hepatocytes establish an infectious HCV murine model. J Clin Invest. 2014;124:4953–64.
18. Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science. 1989;244:359–62.
19. Muchmore E, Popper H, Peterson DA, Miller MF, Lieberman HM. Non-A, non-B hepatitis-related hepatocellular carcinoma in a chimpanzee. J Med Primatol. 1988;17:235–46.
20. Bissig KD, Wieland SF, Tran P, Isogawa M, Le TT, Chisari FV, Verma IM. Human liver chimeric mice provide a model for hepatitis B and C virus infection and treatment. J Clin Invest. 2010;120:924–30.
21. Mercer DF, Schiller DE, Elliott JF, Douglas DN, Hao C, Rinfret A, Addison WR, Fischer KP, Churchill TA, Lakey JR, Tyrrell DL, Kneteman NM. Hepatitis C virus replication in mice with chimeric human livers. Nat Med. 2001;7:927–33.
22. Meuleman P, Libbrecht L, De Vos R, De Hemptinne B, Gevaert K, Vandekerckhove J, Roskams T, Leroux-Roels G. Morphological and biochemical characterization of a human liver in a uPA-SCID mouse chimera. Hepatology. 2005;41:847–56.
23. Calattini S, Fusil F, Mancip J, Dao Thi VL, Granier C, Gadot N, Scoazec JY, Zeisel MB, Baumert TF, Lavillette D, Dreux M, Cosset FL. Functional and biochemical characterization of hepatitis C virus (HCV) particles produced in a humanized liver mouse model. J Biol Chem. 2015;290:23173–87.
24. Steenbergen RH, Joyce MA, Lund G, Lewis J, Chen R, Barsby N, Douglas D, Zhu LF, Tyrrell DL, Kneteman NM. Lipoprotein profiles in SCID/uPA mice transplanted with human

hepatocytes become human-like and correlate with HCV infection success. Am J Physiol Gastrointest Liver Physiol. 2010;299:G844–54.
25. Shi N, Hiraga N, Imamura M, Hayes CN, Zhang Y, Kosaka K, Okazaki A, Murakami E, Tsuge M, Abe H, Aikata H, Takahashi S, Ochi H, Tateno-Mukaidani C, Yoshizato K, Matsui H, Kanai A, Inaba T, McPhee F, Gao M, Chayama K. Combination therapies with NS5A, NS3 and NS5B inhibitors on different genotypes of hepatitis C virus in human hepatocyte chimeric mice. Gut. 2013;62:1055–61.
26. Chen J, Zhao Y, Zhang C, Chen H, Feng J, Chi X, Pan Y, Du J, Guo M, Cao H, Wang Z, Pei R, Wang Q, Pan L, Niu J, Chen X, Tang H. Persistent hepatitis C virus infections and hepatopathological manifestations in immune-competent humanized mice. Cell Res. 2014;24:1050–66.
27. Andre P, Komurian-Pradel F, Deforges S, Perret M, Berland JL, Sodoyer M, Pol S, Brechot C, Paranhos-Baccala G, Lotteau V. Characterization of low- and very-low-density hepatitis C virus RNA-containing particles. J Virol. 2002;76:6919–28.
28. Nielsen SU, Bassendine MF, Burt AD, Martin C, Pumeechockchai W, Toms GL. Association between hepatitis C virus and very-low-density lipoprotein (VLDL)/LDL analyzed in iodixanol density gradients. J Virol. 2006;80:2418–28.
29. Vieyres G, Thomas X, Descamps V, Duverlie G, Patel AH, Dubuisson J. Characterization of the envelope glycoproteins associated with infectious hepatitis C virus. J Virol. 2010;84:10159–68.
30. Maillard P, Walic M, Meuleman P, Roohvand F, Huby T, Le Goff W, Leroux-Roels G, Pecheur EI, Budkowska A. Lipoprotein lipase inhibits hepatitis C virus (HCV) infection by blocking virus cell entry. PLoS One. 2011;6:e26637.
31. Gastaminza P, Dryden KA, Boyd B, Wood MR, Law M, Yeager M, Chisari FV. Ultrastructural and biophysical characterization of hepatitis C virus particles produced in cell culture. J Virol. 2010;84:10999–1009.
32. Merz A, Long G, Hiet MS, Brugger B, Chlanda P, Andre P, Wieland F, Krijnse-Locker J, Bartenschlager R. Biochemical and morphological properties of hepatitis C virus particles and determination of their lipidome. J Biol Chem. 2011;286:3018–32.
33. Aizaki H, Morikawa K, Fukasawa M, Hara H, Inoue Y, Tani H, Saito K, Nishijima M, Hanada K, Matsuura Y, Lai MM, Miyamura T, Wakita T, Suzuki T. Critical role of virion-associated cholesterol and sphingolipid in hepatitis C virus infection. J Virol. 2008;82:5715–24.
34. Felmlee DJ, Sheridan DA, Bridge SH, Nielsen SU, Milne RW, Packard CJ, Caslake MJ, McLauchlan J, Toms GL, Neely RD, Bassendine MF. Intravascular transfer contributes to postprandial increase in numbers of very-low-density hepatitis C virus particles. Gastroenterology. 2010;139:1774-83, 1783 e1-6.
35. Lindenbach BD, Meuleman P, Ploss A, Vanwolleghem T, Syder AJ, McKeating JA, Lanford RE, Feinstone SM, Major ME, Leroux-Roels G, Rice CM. Cell culture grown hepatitis C virus is infectious in vivo and can be recultured in vitro. Proc Natl Acad Sci U S A. 2006;103:3805–9.
36. Lussignol M, Kopp M, Molloy K, Vizcay-Barrena G, Fleck RA, Dorner M, Bell KL, Chait BT, Rice CM, Catanese MT. Proteomics of HCV virions reveals an essential role for the nucleoporin Nup98 in virus morphogenesis. Proc Natl Acad Sci U S A. 2016;113:2484–9.
37. Piver E, Boyer A, Gaillard J, Bull A, Beaumont E, Roingeard P, Meunier JC. Ultrastructural organisation of HCV from the bloodstream of infected patients revealed by electron microscopy after specific immunocapture. Gut. 2016;66(8):1487–95.
38. Scholtes C, Ramiere C, Rainteau D, Perrin-Cocon L, Wolf C, Humbert L, Carreras M, Guironnet-Paquet A, Zoulim F, Bartenschlager R, Lotteau V, Andre P, Diaz O. High plasma level of nucleocapsid-free envelope glycoprotein-positive lipoproteins in hepatitis C patients. Hepatology. 2012;56:39–48.
39. Bankwitz D, Doepke M, Hueging K, Weller R, Bruening J, Behrendt P, Lee JY, Vondran FWR, Manns MP, Bartenschlager R, Pietschmann T. Maturation of secreted HCV particles by

incorporation of secreted ApoE protects from antibodies by enhancing infectivity. J Hepatol. 2017;67:480–9.
40. Santolini E, Migliaccio G, La Monica N. Biosynthesis and biochemical properties of the hepatitis C virus core protein. J Virol. 1994;68:3631–41.
41. Cristofari G, Ivanyi-Nagy R, Gabus C, Boulant S, Lavergne JP, Penin F, Darlix JL. The hepatitis C virus Core protein is a potent nucleic acid chaperone that directs dimerization of the viral (+) strand RNA in vitro. Nucleic Acids Res. 2004;32:2623–31.
42. Boulant S, Montserret R, Hope RG, Ratinier M, Targett-Adams P, Lavergne JP, Penin F, McLauchlan J. Structural determinants that target the hepatitis C virus core protein to lipid droplets. J Biol Chem. 2006;281:22236–47.
43. Murray CL, Jones CT, Tassello J, Rice CM. Alanine scanning of the hepatitis C virus core protein reveals numerous residues essential for production of infectious virus. J Virol. 2007;81:10220–31.
44. Miyanari Y, Atsuzawa K, Usuda N, Watashi K, Hishiki T, Zayas M, Bartenschlager R, Wakita T, Hijikata M, Shimotohno K. The lipid droplet is an important organelle for hepatitis C virus production. Nat Cell Biol. 2007;9:1089–97.
45. Herker E, Harris C, Hernandez C, Carpentier A, Kaehlcke K, Rosenberg AR, Farese RV Jr, Ott M. Efficient hepatitis C virus particle formation requires diacylglycerol acyltransferase-1. Nat Med. 2010;16:1295–8.
46. Camus G, Herker E, Modi AA, Haas JT, Ramage HR, Farese RV Jr, Ott M. Diacylglycerol acyltransferase-1 localizes hepatitis C virus NS5A protein to lipid droplets and enhances NS5A interaction with the viral capsid core. J Biol Chem. 2013;288:9915–23.
47. Vogt DA, Camus G, Herker E, Webster BR, Tsou CL, Greene WC, Yen TS, Ott M. Lipid droplet-binding protein TIP47 regulates hepatitis C Virus RNA replication through interaction with the viral NS5A protein. PLoS Pathog. 2013;9:e1003302.
48. Moradpour D, Penin F, Rice CM. Replication of hepatitis C virus. Nat Rev Microbiol. 2007;5:453–63.
49. Helle F, Vieyres G, Elkrief L, Popescu CI, Wychowski C, Descamps V, Castelain S, Roingeard P, Duverlie G, Dubuisson J. Role of N-linked glycans in the functions of hepatitis C virus envelope proteins incorporated into infectious virions. J Virol. 2010;84:11905–15.
50. Garry RF, Dash S. Proteomics computational analyses suggest that hepatitis C virus E1 and pestivirus E2 envelope glycoproteins are truncated class II fusion proteins. Virology. 2003;307:255–65.
51. El Omari K, Iourin O, Kadlec J, Sutton G, Harlos K, Grimes JM, Stuart DI. Unexpected structure for the N-terminal domain of hepatitis C virus envelope glycoprotein E1. Nat Commun. 2014;5:4874.
52. Jiang J, Cun W, Wu X, Shi Q, Tang H, Luo G. Hepatitis C virus attachment mediated by apolipoprotein E binding to cell surface heparan sulfate. J Virol. 2012;86:7256–67.
53. Mazumdar B, Banerjee A, Meyer K, Ray R. Hepatitis C virus E1 envelope glycoprotein interacts with apolipoproteins in facilitating entry into hepatocytes. Hepatology. 2011;54:1149–56.
54. Falson P, Bartosch B, Alsaleh K, Tews BA, Loquet A, Ciczora Y, Riva L, Montigny C, Montpellier C, Duverlie G, Pecheur EI, Le Maire M, Cosset FL, Dubuisson J, Penin F. Hepatitis C virus envelope glycoprotein E1 forms trimers at the surface of the virion. J Virol. 2015;89:10333–46.
55. Freedman H, Logan MR, Hockman D, Koehler Leman J, Law JL, Houghton M. Computational prediction of the heterodimeric and higher-order structure of gpE1/gpE2 envelope glycoproteins encoded by hepatitis C virus. J Virol. 2017;91:e02309–16.
56. Bartosch B, Vitelli A, Granier C, Goujon C, Dubuisson J, Pascale S, Scarselli E, Cortese R, Nicosia A, Cosset FL. Cell entry of hepatitis C virus requires a set of co-receptors that include the CD81 tetraspanin and the SR-B1 scavenger receptor. J Biol Chem. 2003;278:41624–30.

57. Scarselli E, Ansuini H, Cerino R, Roccasecca RM, Acali S, Filocamo G, Traboni C, Nicosia A, Cortese R, Vitelli A. The human scavenger receptor class B type I is a novel candidate receptor for the hepatitis C virus. EMBO J. 2002;21:5017–25.
58. Farci P, Shimoda A, Wong D, Cabezon T, De Gioannis D, Strazzera A, Shimizu Y, Shapiro M, Alter HJ, Purcell RH. Prevention of hepatitis C virus infection in chimpanzees by hyperimmune serum against the hypervariable region 1 of the envelope 2 protein. Proc Natl Acad Sci U S A. 1996;93:15394–9.
59. Bankwitz D, Vieyres G, Hueging K, Bitzegeio J, Doepke M, Chhatwal P, Haid S, Catanese MT, Zeisel MB, Nicosia A, Baumert TF, Kaderali L, Pietschmann T. Role of hypervariable region 1 for the interplay of hepatitis C virus with entry factors and lipoproteins. J Virol. 2014;88:12644–55.
60. Bartosch B, Verney G, Dreux M, Donot P, Morice Y, Penin F, Pawlotsky JM, Lavillette D, Cosset FL. An interplay between hypervariable region 1 of the hepatitis C virus E2 glycoprotein, the scavenger receptor BI, and high-density lipoprotein promotes both enhancement of infection and protection against neutralizing antibodies. J Virol. 2005;79:8217–29.
61. Liu L, Fisher BE, Thomas DL, Cox AL, Ray SC. Spontaneous clearance of primary acute hepatitis C virus infection correlated with high initial viral RNA level and rapid HVR1 evolution. Hepatology. 2012;55:1684–91.
62. Bankwitz D, Steinmann E, Bitzegeio J, Ciesek S, Friesland M, Herrmann E, Zeisel MB, Baumert TF, Keck ZY, Foung SK, Pecheur EI, Pietschmann T. Hepatitis C virus hypervariable region 1 modulates receptor interactions, conceals the CD81 binding site, and protects conserved neutralizing epitopes. J Virol. 2010;84:5751–63.
63. Modis Y, Ogata S, Clements D, Harrison SC. A ligand-binding pocket in the dengue virus envelope glycoprotein. Proc Natl Acad Sci U S A. 2003;100:6986–91.
64. Modis Y, Ogata S, Clements D, Harrison SC. Structure of the dengue virus envelope protein after membrane fusion. Nature. 2004;427:313–9.
65. Bressanelli S, Stiasny K, Allison SL, Stura EA, Duquerroy S, Lescar J, Heinz FX, Rey FA. Structure of a flavivirus envelope glycoprotein in its low-pH-induced membrane fusion conformation. EMBO J. 2004;23:728–38.
66. Lescar J, Roussel A, Wien MW, Navaza J, Fuller SD, Wengler G, Wengler G, Rey FA. The Fusion glycoprotein shell of Semliki Forest virus: an icosahedral assembly primed for fusogenic activation at endosomal pH. Cell. 2001;105:137–48.
67. Krey T, D'alayer J, Kikuti CM, Saulnier A, Damier-Piolle L, Petitpas I, Johansson DX, Tawar RG, Baron B, Robert B, England P, Persson MA, Martin A, Rey FA. The disulfide bonds in glycoprotein E2 of hepatitis C virus reveal the tertiary organization of the molecule. PLoS Pathog. 2010;6:e1000762.
68. Lavillette D, Bartosch B, Nourrisson D, Verney G, Cosset FL, Penin F, Pecheur EI. Hepatitis C virus glycoproteins mediate low pH-dependent membrane fusion with liposomes. J Biol Chem. 2006;281:3909–17.
69. Khan AG, Whidby J, Miller MT, Scarborough H, Zatorski AV, Cygan A, Price AA, Yost SA, Bohannon CD, Jacob J, Grakoui A, Marcotrigiano J. Structure of the core ectodomain of the hepatitis C virus envelope glycoprotein 2. Nature. 2014;509:381–4.
70. Kong L, Giang E, Nieusma T, Kadam RU, Cogburn KE, Hua Y, Dai X, Stanfield RL, Burton DR, Ward AB, Wilson IA, Law M. Hepatitis C virus E2 envelope glycoprotein core structure. Science. 2013;342:1090–4.
71. Vaney MC, Rey FA. Class II enveloped viruses. Cell Microbiol. 2011;13:1451–9.
72. Boyer A, Dumans A, Beaumont E, Etienne L, Roingeard P, Meunier JC. The association of hepatitis C virus glycoproteins with apolipoproteins E and B early in assembly is conserved in lipoviral particles. J Biol Chem. 2014;289:18904–13.
73. Gentzsch J, Brohm C, Steinmann E, Friesland M, Menzel N, Vieyres G, Perin PM, Frentzen A, Kaderali L, Pietschmann T. hepatitis c virus p7 is critical for capsid assembly and envelopment. PLoS Pathog. 2013;9:e1003355.

74. Griffin SD, Beales LP, Clarke DS, Worsfold O, Evans SD, Jaeger J, Harris MP, Rowlands DJ. The p7 protein of hepatitis C virus forms an ion channel that is blocked by the antiviral drug, Amantadine. FEBS Lett. 2003;535:34–8.
75. Chandler DE, Penin F, Schulten K, Chipot C. The p7 protein of hepatitis C virus forms structurally plastic, minimalist ion channels. PLoS Comput Biol. 2012;8:e1002702.
76. Wozniak AL, Griffin S, Rowlands D, Harris M, Yi M, Lemon SM, Weinman SA. Intracellular proton conductance of the hepatitis C virus p7 protein and its contribution to infectious virus production. PLoS Pathog. 2010;6:e1001087.
77. Boson B, Granio O, Bartenschlager R, Cosset FL. A concerted action of hepatitis C virus p7 and nonstructural protein 2 regulates core localization at the endoplasmic reticulum and virus assembly. PLoS Pathog. 2011;7:e1002144.
78. Hagen N, Bayer K, Rosch K, Schindler M. The intraviral protein interaction network of hepatitis C virus. Mol Cell Proteomics. 2014;13:1676–89.
79. Jirasko V, Montserret R, Lee JY, Gouttenoire J, Moradpour D, Penin F, Bartenschlager R. Structural and functional studies of nonstructural protein 2 of the hepatitis C virus reveal its key role as organizer of virion assembly. PLoS Pathog. 2010;6:e1001233.
80. Tomei L, Failla C, Santolini E, De Francesco R, La Monica N. NS3 is a serine protease required for processing of hepatitis C virus polyprotein. J Virol. 1993;67:4017–26.
81. Lorenz IC, Marcotrigiano J, Dentzer TG, Rice CM. Structure of the catalytic domain of the hepatitis C virus NS2-3 protease. Nature. 2006;442:831–5.
82. Reed KE, Grakoui A, Rice CM. Hepatitis C virus-encoded NS2-3 protease: cleavage-site mutagenesis and requirements for bimolecular cleavage. J Virol. 1995;69:4127–36.
83. Grakoui A, Wychowski C, Lin C, Feinstone SM, Rice CM. Expression and identification of hepatitis C virus polyprotein cleavage products. J Virol. 1993;67:1385–95.
84. Welbourn S, Jirasko V, Breton V, Reiss S, Penin F, Bartenschlager R, Pause A. Investigation of a role for lysine residues in non-structural proteins 2 and 2/3 of the hepatitis C virus for their degradation and virus assembly. J Gen Virol. 2009;90:1071–80.
85. Grakoui A, McCourt DW, Wychowski C, Feinstone SM, Rice CM. A second hepatitis C virus-encoded proteinase. Proc Natl Acad Sci U S A. 1993;90:10583–7.
86. Hijikata M, Mizushima H, Akagi T, Mori S, Kakiuchi N, Kato N, Tanaka T, Kimura K, Shimotohno K. Two distinct proteinase activities required for the processing of a putative nonstructural precursor protein of hepatitis C virus. J Virol. 1993;67:4665–75.
87. Yi M, Ma Y, Yates J, Lemon SM. Trans-complementation of an NS2 defect in a late step in hepatitis C virus (HCV) particle assembly and maturation. PLoS Pathog. 2009;5:e1000403.
88. Foy E, Li K, Wang C, Sumpter R Jr, Ikeda M, Lemon SM, Gale M Jr. Regulation of interferon regulatory factor-3 by the hepatitis C virus serine protease. Science. 2003;300:1145–8.
89. Jones CT, Murray CL, Eastman DK, Tassello J, Rice CM. Hepatitis C virus p7 and NS2 proteins are essential for production of infectious virus. J Virol. 2007;81:8374–83.
90. Kolykhalov AA, Mihalik K, Feinstone SM, Rice CM. Hepatitis C virus-encoded enzymatic activities and conserved RNA elements in the 3' nontranslated region are essential for virus replication in vivo. J Virol. 2000;74:2046–51.
91. Schregel V, Jacobi S, Penin F, Tautz N. Hepatitis C virus NS2 is a protease stimulated by cofactor domains in NS3. Proc Natl Acad Sci U S A. 2009;106:5342–7.
92. Jirasko V, Montserret R, Appel N, Janvier A, Eustachi L, Brohm C, Steinmann E, Pietschmann T, Penin F, Bartenschlager R. Structural and functional characterization of nonstructural protein 2 for its role in hepatitis C virus assembly. J Biol Chem. 2008;283:28546–62.
93. Pietschmann T, Kaul A, Koutsoudakis G, Shavinskaya A, Kallis S, Steinmann E, Abid K, Negro F, Dreux M, Cosset FL, Bartenschlager R. Construction and characterization of infectious intragenotypic and intergenotypic hepatitis C virus chimeras. Proc Natl Acad Sci U S A. 2006;103:7408–13.

94. Bittar C, Shrivastava S, Bhanja Chowdhury J, Rahal P, Ray RB. Hepatitis C virus NS2 protein inhibits DNA damage pathway by sequestering p53 to the cytoplasm. PLoS One. 2013;8: e62581.
95. Erdtmann L, Franck N, Lerat H, Le Seyec J, Gilot D, Cannie I, Gripon P, Hibner U, Guguen-Guillouzo C. The hepatitis C virus NS2 protein is an inhibitor of CIDE-B-induced apoptosis. J Biol Chem. 2003;278:18256–64.
96. Kim KM, Kwon SN, Kang JI, Lee SH, Jang SK, Ahn BY, Kim YK. Hepatitis C virus NS2 protein activates cellular cyclic AMP-dependent pathways. Biochem Biophys Res Commun. 2007;356:948–54.
97. Oem JK, Jackel-Cram C, Li YP, Kang HN, Zhou Y, Babiuk LA, Liu Q. Hepatitis C virus non-structural protein-2 activates CXCL-8 transcription through NF-kappaB. Arch Virol. 2008;153:293–301.
98. Oem JK, Jackel-Cram C, Li YP, Zhou Y, Zhong J, Shimano H, Babiuk LA, Liu Q. Activation of sterol regulatory element-binding protein 1c and fatty acid synthase transcription by hepatitis C virus non-structural protein 2. J Gen Virol. 2008;89:1225–30.
99. Yang XJ, Liu J, Ye L, Liao QJ, Wu JG, Gao JR, She YL, Wu ZH, Ye LB. HCV NS2 protein inhibits cell proliferation and induces cell cycle arrest in the S-phase in mammalian cells through down-regulation of cyclin A expression. Virus Res. 2006;121:134–43.
100. Yao N, Reichert P, Taremi SS, Prosise WW, Weber PC. Molecular views of viral polyprotein processing revealed by the crystal structure of the hepatitis C virus bifunctional protease-helicase. Structure. 1999;7:1353–63.
101. Lindenbach BD, Pragai BM, Montserret R, Beran RK, Pyle AM, Penin F, Rice CM. The C terminus of hepatitis C virus NS4A encodes an electrostatic switch that regulates NS5A hyperphosphorylation and viral replication. J Virol. 2007;81:8905–18.
102. Morikawa K, Lange CM, Gouttenoire J, Meylan E, Brass V, Penin F, Moradpour D. Nonstructural protein 3-4A: the Swiss army knife of hepatitis C virus. J Viral Hepat. 2011;18:305–15.
103. Horner SM, Liu HM, Park HS, Briley J, Gale M Jr. Mitochondrial-associated endoplasmic reticulum membranes (MAM) form innate immune synapses and are targeted by hepatitis C virus. Proc Natl Acad Sci U S A. 2011;108:14590–5.
104. Wolk B, Sansonno D, Krausslich HG, Dammacco F, Rice CM, Blum HE, Moradpour D. Subcellular localization, stability, and trans-cleavage competence of the hepatitis C virus NS3-NS4A complex expressed in tetracycline-regulated cell lines. J Virol. 2000;74:2293–304.
105. Meylan E, Curran J, Hofmann K, Moradpour D, Binder M, Bartenschlager R, Tschopp J. Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. Nature. 2005;437:1167–72.
106. Gagne B, Tremblay N, Park AY, Baril M, Lamarre D. Importin beta1 targeting by hepatitis C virus NS3/4A protein restricts IRF3 and NF-kappaB signaling of IFNB1 antiviral response. Traffic. 2017;18:362–77.
107. Yu GY, Lee KJ, Gao L, Lai MM. Palmitoylation and polymerization of hepatitis C virus NS4B protein. J Virol. 2006;80:6013–23.
108. Lundin M, Monne M, Widell A, Von Heijne G, Persson MA. Topology of the membrane-associated hepatitis C virus protein NS4B. J Virol. 2003;77:5428–38.
109. Gouttenoire J, Roingeard P, Penin F, Moradpour D. Amphipathic alpha-helix AH2 is a major determinant for the oligomerization of hepatitis C virus nonstructural protein 4B. J Virol. 2010;84:12529–37.
110. Gouttenoire J, Montserret R, Paul D, Castillo R, Meister S, Bartenschlager R, Penin F, Moradpour D. Aminoterminal amphipathic alpha-helix AH1 of hepatitis C virus nonstructural protein 4B possesses a dual role in RNA replication and virus production. PLoS Pathog. 2014;10:e1004501.
111. Gouttenoire J, Castet V, Montserret R, Arora N, Raussens V, Ruysschaert JM, Diesis E, Blum HE, Penin F, Moradpour D. Identification of a novel determinant for membrane association in hepatitis C virus nonstructural protein 4B. J Virol. 2009;83:6257–68.

112. Egger D, Wolk B, Gosert R, Bianchi L, Blum HE, Moradpour D, Bienz K. Expression of hepatitis C virus proteins induces distinct membrane alterations including a candidate viral replication complex. J Virol. 2002;76:5974–84.
113. Gosert R, Egger D, Lohmann V, Bartenschlager R, Blum HE, Bienz K, Moradpour D. Identification of the hepatitis C virus RNA replication complex in Huh-7 cells harboring subgenomic replicons. J Virol. 2003;77:5487–92.
114. Ashworth Briggs EL, Gomes RG, Elhussein M, Collier W, Findlow IS, Khalid S, McCormick CJ, Williamson PT. Interaction between the NS4B amphipathic helix, AH2, and charged lipid headgroups alters membrane morphology and AH2 oligomeric state—Implications for the Hepatitis C virus life cycle. Biochim Biophys Acta. 2015;1848:1671–7.
115. Kong L, Fujimoto A, Nakamura M, Aoyagi H, Matsuda M, Watashi K, Suzuki R, Arita M, Yamagoe S, Dohmae N, Suzuki T, Sakamaki Y, Ichinose S, Wakita T, Aizaki H. Prolactin regulatory element binding protein is involved in hepatitis C virus replication by interaction with NS4B. J Virol. 2016;90:3093–111.
116. Biswas A, Treadaway J, Tellinghuisen TL. Interaction between nonstructural proteins NS4B and NS5A Is essential for proper NS5A localization and hepatitis C virus RNA replication. J Virol. 2016;90:7205–18.
117. Quinkert D, Bartenschlager R, Lohmann V. Quantitative analysis of the hepatitis C virus replication complex. J Virol. 2005;79:13594–605.
118. Romero-Brey I, Berger C, Kallis S, Kolovou A, Paul D, Lohmann V, Bartenschlager R. NS5A domain 1 and polyprotein cleavage kinetics are critical for induction of double-membrane vesicles associated with hepatitis C virus replication. MBio. 2015;6:e00759.
119. Einav S, Sklan EH, Moon HM, Gehrig E, Liu P, Hao Y, Lowe AW, Glenn JS. The nucleotide binding motif of hepatitis C virus NS4B can mediate cellular transformation and tumor formation without Ha-ras co-transfection. Hepatology. 2008;47:827–35.
120. Jones DM, Patel AH, Targett-Adams P, McLauchlan J. The hepatitis C virus NS4B protein can trans-complement viral RNA replication and modulates production of infectious virus. J Virol. 2009;83:2163–77.
121. Romero-Brey I, Merz A, Chiramel A, Lee JY, Chlanda P, Haselman U, Santarella-Mellwig R, Habermann A, Hoppe S, Kallis S, Walther P, Antony C, Krijnse-Locker J, Bartenschlager R. Three-dimensional architecture and biogenesis of membrane structures associated with hepatitis C virus replication. PLoS Pathog. 2012;8:e1003056.
122. Elazar M, Cheong KH, Liu P, Greenberg HB, Rice CM, Glenn JS. Amphipathic helix-dependent localization of NS5A mediates hepatitis C virus RNA replication. J Virol. 2003;77:6055–61.
123. Moradpour D, Brass V, Penin F. Function follows form: the structure of the N-terminal domain of HCV NS5A. Hepatology. 2005;42:732–5.
124. Foster TL, Belyaeva T, Stonehouse NJ, Pearson AR, Harris M. All three domains of the hepatitis C virus nonstructural NS5A protein contribute to RNA binding. J Virol. 2010;84:9267–77.
125. Huang L, Hwang J, Sharma SD, Hargittai MR, Chen Y, Arnold JJ, Raney KD, Cameron CE. Hepatitis C virus nonstructural protein 5A (NS5A) is an RNA-binding protein. J Biol Chem. 2005;280:36417–28.
126. Tellinghuisen TL, Marcotrigiano J, Gorbalenya AE, Rice CM. The NS5A protein of hepatitis C virus is a zinc metalloprotein. J Biol Chem. 2004;279:48576–87.
127. Love RA, Brodsky O, Hickey MJ, Wells PA, Cronin CN. Crystal structure of a novel dimeric form of NS5A domain I protein from hepatitis C virus. J Virol. 2009;83:4395–403.
128. Tellinghuisen TL, Marcotrigiano J, Rice CM. Structure of the zinc-binding domain of an essential component of the hepatitis C virus replicase. Nature. 2005;435:374–9.
129. Hanoulle X, Verdegem D, Badillo A, Wieruszeski JM, Penin F, Lippens G. Domain 3 of non-structural protein 5A from hepatitis C virus is natively unfolded. Biochem Biophys Res Commun. 2009;381:634–8.

130. Liang Y, Ye H, Kang CB, Yoon HS. Domain 2 of nonstructural protein 5A (NS5A) of hepatitis C virus is natively unfolded. Biochemistry. 2007;46:11550–8.
131. Appel N, Zayas M, Miller S, Krijnse-Locker J, Schaller T, Friebe P, Kallis S, Engel U, Bartenschlager R. Essential role of domain III of nonstructural protein 5A for hepatitis C virus infectious particle assembly. PLoS Pathog. 2008;4:e1000035.
132. Ross-Thriepland D, Amako Y, Harris M. The C terminus of NS5A domain II is a key determinant of hepatitis C virus genome replication, but is not required for virion assembly and release. J Gen Virol. 2013;94:1009–18.
133. Tellinghuisen TL, Foss KL, Treadaway JC, Rice CM. Identification of residues required for RNA replication in domains II and III of the hepatitis C virus NS5A protein. J Virol. 2008;82:1073–83.
134. Appel N, Pietschmann T, Bartenschlager R. Mutational analysis of hepatitis C virus nonstructural protein 5A: potential role of differential phosphorylation in RNA replication and identification of a genetically flexible domain. J Virol. 2005;79:3187–94.
135. Huang Y, Staschke K, De Francesco R, Tan SL. Phosphorylation of hepatitis C virus NS5A nonstructural protein: a new paradigm for phosphorylation-dependent viral RNA replication? Virology. 2007;364:1–9.
136. Tanji Y, Kaneko T, Satoh S, Shimotohno K. Phosphorylation of hepatitis C virus-encoded nonstructural protein NS5A. J Virol. 1995;69:3980–6.
137. MacDonald A, Harris M. Hepatitis C virus NS5A: tales of a promiscuous protein. J Gen Virol. 2004;85:2485–502.
138. Ahn J, Chung KS, Kim DU, Won M, Kim L, Kim KS, Nam M, Choi SJ, Kim HC, Yoon M, Chae SK, Hoe KL. Systematic identification of hepatocellular proteins interacting with NS5A of the hepatitis C virus. J Biochem Mol Biol. 2004;37:741–8.
139. De Chassey B, Navratil V, Tafforeau L, Hiet MS, Aublin-Gex A, Agaugue S, Meiffren G, Pradezynski F, Faria BF, Chantier T, Le Breton M, Pellet J, Davoust N, Mangeot PE, Chaboud A, Penin F, Jacob Y, Vidalain PO, Vidal M, Andre P, Rabourdin-Combe C, Lotteau V. Hepatitis C virus infection protein network. Mol Syst Biol. 2008;4:230.
140. Wang H, Perry JW, Lauring AS, Neddermann P, De Francesco R, Tai AW. Oxysterol-binding protein is a phosphatidylinositol 4-kinase effector required for HCV replication membrane integrity and cholesterol trafficking. Gastroenterology. 2014;146(1373-85):e1–11.
141. Reiss S, Harak C, Romero-Brey I, Radujkovic D, Klein R, Ruggieri A, Rebhan I, Bartenschlager R, Lohmann V. The lipid kinase phosphatidylinositol-4 kinase III alpha regulates the phosphorylation status of hepatitis C virus NS5A. PLoS Pathog. 2013;9:e1003359.
142. Majumder M, Ghosh AK, Steele R, Ray R, Ray RB. Hepatitis C virus NS5A physically associates with p53 and regulates p21/waf1 gene expression in a p53-dependent manner. J Virol. 2001;75:1401–7.
143. Arima N, Kao CY, Licht T, Padmanabhan R, Sasaguri Y. Modulation of cell growth by the hepatitis C virus nonstructural protein NS5A. J Biol Chem. 2001;276:12675–84.
144. Tan SL, Nakao H, He Y, Vijaysri S, Neddermann P, Jacobs BL, Mayer BJ, Katze MG. NS5A, a nonstructural protein of hepatitis C virus, binds growth factor receptor-bound protein 2 adaptor protein in a Src homology 3 domain/ligand-dependent manner and perturbs mitogenic signaling. Proc Natl Acad Sci U S A. 1999;96:5533–8.
145. Chung YL, Sheu ML, Yen SH. Hepatitis C virus NS5A as a potential viral Bcl-2 homologue interacts with Bax and inhibits apoptosis in hepatocellular carcinoma. Int J Cancer. 2003;107:65–73.
146. MacDonald A, Chan JK, Harris M. Perturbation of epidermal growth factor receptor complex formation and Ras signalling in cells harbouring the hepatitis C virus subgenomic replicon. J Gen Virol. 2005;86:1027–33.
147. Mankouri J, Griffin S, Harris M. The hepatitis C virus non-structural protein NS5A alters the trafficking profile of the epidermal growth factor receptor. Traffic. 2008;9:1497–509.

148. Shi ST, Polyak SJ, Tu H, Taylor DR, Gretch DR, Lai MM. Hepatitis C virus NS5A colocalizes with the core protein on lipid droplets and interacts with apolipoproteins. Virology. 2002;292:198–210.
149. Domitrovich AM, Felmlee DJ, Siddiqui A. Hepatitis C virus nonstructural proteins inhibit apolipoprotein B100 secretion. J Biol Chem. 2005;280:39802–8.
150. Lesburg CA, Cable MB, Ferrari E, Hong Z, Mannarino AF, Weber PC. Crystal structure of the RNA-dependent RNA polymerase from hepatitis C virus reveals a fully encircled active site. Nat Struct Biol. 1999;6:937–43.
151. Sesmero E, Thorpe IF. Using the hepatitis C virus RNA-dependent RNA polymerase as a model to understand viral polymerase structure, function and dynamics. Viruses. 2015;7:3974–94.
152. Wang QM, Hockman MA, Staschke K, Johnson RB, Case KA, Lu J, Parsons S, Zhang F, Rathnachalam R, Kirkegaard K, Colacino JM. Oligomerization and cooperative RNA synthesis activity of hepatitis C virus RNA-dependent RNA polymerase. J Virol. 2002;76:3865–72.
153. Moradpour D, Brass V, Bieck E, Friebe P, Gosert R, Blum HE, Bartenschlager R, Penin F, Lohmann V. Membrane association of the RNA-dependent RNA polymerase is essential for hepatitis C virus RNA replication. J Virol. 2004;78:13278–84.
154. Aligeti M, Roder A, Horner SM. Cooperation between the Hepatitis C Virus p7 and NS5B Proteins Enhances Virion Infectivity. J Virol. 2015;89:11523–33.
155. Bartosch B, Dubuisson J, Cosset FL. Infectious hepatitis C virus pseudo-particles containing functional E1-E2 envelope protein complexes. J Exp Med. 2003;197:633–42.
156. Op De Beeck A, Voisset C, Bartosch B, Ciczora Y, Cocquerel L, Keck Z, Foung S, Cosset FL, Dubuisson J. Characterization of functional hepatitis C virus envelope glycoproteins. J Virol. 2004;78:2994–3002.
157. Sandrin V, Boulanger P, Penin F, Granier C, Cosset FL, Bartosch B. Assembly of functional hepatitis C virus glycoproteins on infectious pseudoparticles occurs intracellularly and requires concomitant incorporation of E1 and E2 glycoproteins. J Gen Virol. 2005;86:3189–99.
158. Fan H, Qiao L, Kang KD, Fan J, Wei W, Luo G. Attachment and postattachment receptors important for hepatitis C virus infection and cell-to-cell transmission. J Virol. 2017;91: e00280-17.
159. Pileri P, Uematsu Y, Campagnoli S, Galli G, Falugi F, Petracca R, Weiner AJ, Houghton M, Rosa D, Grandi G, Abrignani S. Binding of hepatitis C virus to CD81. Science. 1998;282:938–41.
160. Dreux M, Dao Thi VL, Fresquet J, Guerin M, Julia Z, Verney G, Durantel D, Zoulim F, Lavillette D, Cosset FL, Bartosch B. Receptor complementation and mutagenesis reveal SR-BI as an essential HCV entry factor and functionally imply its intra- and extra-cellular domains. PLoS Pathog. 2009;5:e1000310.
161. Dao Thi VL, Granier C, Zeisel MB, Guerin M, Mancip J, Granio O, Penin F, Lavillette D, Bartenschlager R, Baumert TF, Cosset FL, Dreux M. Characterization of hepatitis C virus particle subpopulations reveals multiple usage of the scavenger receptor BI for entry steps. J Biol Chem. 2012;287:31242–57.
162. Agnello V, Abel G, Elfahal M, Knight GB, Zhang QX. Hepatitis C virus and other flaviviridae viruses enter cells via low density lipoprotein receptor. Proc Natl Acad Sci U S A. 1999;96:12766–71.
163. Molina S, Castet V, Fournier-Wirth C, Pichard-Garcia L, Avner R, Harats D, Roitelman J, Barbaras R, Graber P, Ghersa P, Smolarsky M, Funaro A, Malavasi F, Larrey D, Coste J, Fabre JM, Sa-Cunha A, Maurel P. The low-density lipoprotein receptor plays a role in the infection of primary human hepatocytes by hepatitis C virus. J Hepatol. 2007;46:411–9.
164. Albecka A, Belouzard S, Op De Beeck A, Descamps V, Goueslain L, Bertrand-Michel J, Terce F, Duverlie G, Rouille Y, Dubuisson J. Role of low-density lipoprotein receptor in the hepatitis C virus life cycle. Hepatology. 2012;55:998–1007.

165. Levy S, Shoham T. Protein-protein interactions in the tetraspanin web. Physiology (Bethesda). 2005;20:218–24.
166. Petracca R, Falugi F, Galli G, Norais N, Rosa D, Campagnoli S, Burgio V, Di Stasio E, Giardina B, Houghton M, Abrignani S, Grandi G. Structure-function analysis of hepatitis C virus envelope-CD81 binding. J Virol. 2000;74:4824–30.
167. Benedicto I, Molina-Jimenez F, Bartosch B, Cosset FL, Lavillette D, Prieto J, Moreno-Otero R, Valenzuela-Fernandez A, Aldabe R, Lopez-Cabrera M, Majano PL. The tight junction-associated protein occludin is required for a postbinding step in hepatitis C virus entry and infection. J Virol. 2009;83:8012–20.
168. Harris HJ, Davis C, Mullins JG, Hu K, Goodall M, Farquhar MJ, Mee CJ, McCaffrey K, Young S, Drummer H, Balfe P, McKeating JA. Claudin association with CD81 defines hepatitis C virus entry. J Biol Chem. 2010;285:21092–102.
169. Yin P, Li Y, Zhang L. Sec24C-dependent transport of claudin-1 regulates hepatitis C virus entry. J Virol. 2017;91:e00629–17.
170. Tscherne DM, Evans MJ, Von Hahn T, Jones CT, Stamataki Z, McKeating JA, Lindenbach BD, Rice CM. Superinfection exclusion in cells infected with hepatitis C virus. J Virol. 2007;81:3693–703.
171. Zona L, Lupberger J, Sidahmed-Adrar N, Thumann C, Harris HJ, Barnes A, Florentin J, Tawar RG, Xiao F, Turek M, Durand SC, Duong FH, Heim MH, Cosset FL, Hirsch I, Samuel D, Brino L, Zeisel MB, Le Naour F, McKeating JA, Baumert TF. HRas signal transduction promotes hepatitis C virus cell entry by triggering assembly of the host tetraspanin receptor complex. Cell Host Microbe. 2013;13:302–13.
172. Ujino S, Nishitsuji H, Hishiki T, Sugiyama K, Takaku H, Shimotohno K. Hepatitis C virus utilizes VLDLR as a novel entry pathway. Proc Natl Acad Sci U S A. 2016;113:188–93.
173. Zeisel MB, Koutsoudakis G, Schnober EK, Haberstroh A, Blum HE, Cosset FL, Wakita T, Jaeck D, Doffoel M, Royer C, Soulier E, Schvoerer E, Schuster C, Stoll-Keller F, Bartenschlager R, Pietschmann T, Barth H, Baumert TF. Scavenger receptor class B type I is a key host factor for hepatitis C virus infection required for an entry step closely linked to CD81. Hepatology. 2007;46:1722–31.
174. Blaising J, Levy PL, Gondeau C, Phelip C, Varbanov M, Teissier E, Ruggiero F, Polyak SJ, Oberlies NH, Ivanovic T, Boulant S, Pecheur EI. Silibinin inhibits hepatitis C virus entry into hepatocytes by hindering clathrin-dependent trafficking. Cell Microbiol. 2013;15:1866–82.
175. Blanchard E, Belouzard S, Goueslain L, Wakita T, Dubuisson J, Wychowski C, Rouille Y. Hepatitis C virus entry depends on clathrin-mediated endocytosis. J Virol. 2006;80:6964–72.
176. Stamataki Z, Shannon-Lowe C, Shaw J, Mutimer D, Rickinson AB, Gordon J, Adams DH, Balfe P, McKeating JA. Hepatitis C virus association with peripheral blood B lymphocytes potentiates viral infection of liver-derived hepatoma cells. Blood. 2009;113:585–93.
177. Chen CL, Huang JY, Wang CH, Tahara SM, Zhou L, Kondo Y, Schechter J, Su L, Lai MM, Wakita T, Cosset FL, Jung JU, Machida K. Hepatitis C virus has a genetically determined lymphotropism through co-receptor B7.2. Nat Commun. 2017;8:13882.
178. Brimacombe CL, Grove J, Meredith LW, Hu K, Syder AJ, Flores MV, Timpe JM, Krieger SE, Baumert TF, Tellinghuisen TL, Wong-Staal F, Balfe P, McKeating JA. Neutralizing antibody-resistant hepatitis C virus cell-to-cell transmission. J Virol. 2011;85:596–605.
179. Timpe JM, Stamataki Z, Jennings A, Hu K, Farquhar MJ, Harris HJ, Schwarz A, Desombere I, Roels GL, Balfe P, McKeating JA. Hepatitis C virus cell-cell transmission in hepatoma cells in the presence of neutralizing antibodies. Hepatology. 2008;47:17–24.
180. Witteveldt J, Evans MJ, Bitzegeio J, Koutsoudakis G, Owsianka AM, Angus AG, Keck ZY, Foung SK, Pietschmann T, Rice CM, Patel AH. CD81 is dispensable for hepatitis C virus cell-to-cell transmission in hepatoma cells. J Gen Virol. 2009;90:48–58.
181. Barretto N, Sainz B Jr, Hussain S, Uprichard SL. Determining the involvement and therapeutic implications of host cellular factors in hepatitis C virus cell-to-cell spread. J Virol. 2014;88:5050–61.

182. Hueging K, Doepke M, Vieyres G, Bankwitz D, Frentzen A, Doerrbecker J, Gumz F, Haid S, Wolk B, Kaderali L, Pietschmann T. Apolipoprotein E codetermines tissue tropism of hepatitis C virus and is crucial for viral cell-to-cell transmission by contributing to a postenvelopment step of assembly. J Virol. 2014;88:1433–46.
183. Catanese MT, Loureiro J, Jones CT, Dorner M, Von Hahn T, Rice CM. Different requirements for scavenger receptor class B type I in hepatitis C virus cell-free versus cell-to-cell transmission. J Virol. 2013;87:8282–93.
184. Haid S, Pietschmann T, Pecheur EI. Low pH-dependent hepatitis C virus membrane fusion depends on E2 integrity, target lipid composition, and density of virus particles. J Biol Chem. 2009;284:17657–67.
185. Sharma NR, Mateu G, Dreux M, Grakoui A, Cosset FL, Melikyan GB. Hepatitis C virus is primed by CD81 protein for low pH-dependent fusion. J Biol Chem. 2011;286:30361–76.
186. Kim SJ, Kim JH, Kim YG, Lim HS, Oh JW. Protein kinase C-related kinase 2 regulates hepatitis C virus RNA polymerase function by phosphorylation. J Biol Chem. 2004;279:50031–41.
187. Inoue Y, Aizaki H, Hara H, Matsuda M, Ando T, Shimoji T, Murakami K, Masaki T, Shoji I, Homma S, Matsuura Y, Miyamura T, Wakita T, Suzuki T. Chaperonin TRiC/CCT participates in replication of hepatitis C virus genome via interaction with the viral NS5B protein. Virology. 2011;410:38–47.
188. Dimitrova M, Imbert I, Kieny MP, Schuster C. Protein-protein interactions between hepatitis C virus nonstructural proteins. J Virol. 2003;77:5401–14.
189. Gouttenoire J, Penin F, Moradpour D. Hepatitis C virus nonstructural protein 4B: a journey into unexplored territory. Rev Med Virol. 2010;20:117–29.
190. Paredes AM, Blight KJ. A genetic interaction between hepatitis C virus NS4B and NS3 is important for RNA replication. J Virol. 2008;82:10671–83.
191. Piccininni S, Varaklioti A, Nardelli M, Dave B, Raney KD, McCarthy JE. Modulation of the hepatitis C virus RNA-dependent RNA polymerase activity by the non-structural (NS) 3 helicase and the NS4B membrane protein. J Biol Chem. 2002;277:45670–9.
192. Binder M, Quinkert D, Bochkarova O, Klein R, Kezmic N, Bartenschlager R, Lohmann V. Identification of determinants involved in initiation of hepatitis C virus RNA synthesis by using intergenotypic replicase chimeras. J Virol. 2007;81:5270–83.
193. Reigadas S, Ventura M, Sarih-Cottin L, Castroviejo M, Litvak S, Astier-Gin T. HCV RNA-dependent RNA polymerase replicates in vitro the 3' terminal region of the minus-strand viral RNA more efficiently than the 3' terminal region of the plus RNA. Eur J Biochem. 2001;268:5857–67.
194. Harrus D, Ahmed-El-Sayed N, Simister PC, Miller S, Triconnet M, Hagedorn CH, Mahias K, Rey FA, Astier-Gin T, Bressanelli S. Further insights into the roles of GTP and the C terminus of the hepatitis C virus polymerase in the initiation of RNA synthesis. J Biol Chem. 2010;285:32906–18.
195. Ferrari E, He Z, Palermo RE, Huang HC. Hepatitis C virus NS5B polymerase exhibits distinct nucleotide requirements for initiation and elongation. J Biol Chem. 2008;283:33893–901.
196. Biswal BK, Cherney MM, Wang M, Chan L, Yannopoulos CG, Bilimoria D, Nicolas O, Bedard J, James MN. Crystal structures of the RNA-dependent RNA polymerase genotype 2a of hepatitis C virus reveal two conformations and suggest mechanisms of inhibition by non-nucleoside inhibitors. J Biol Chem. 2005;280:18202–10.
197. Mosley RT, Edwards TE, Murakami E, Lam AM, Grice RL, Du J, Sofia MJ, Furman PA, Otto MJ. Structure of hepatitis C virus polymerase in complex with primer-template RNA. J Virol. 2012;86:6503–11.
198. Powdrill MH, Tchesnokov EP, Kozak RA, Russell RS, Martin R, Svarovskaia ES, Mo H, Kouyos RD, Gotte M. Contribution of a mutational bias in hepatitis C virus replication to the genetic barrier in the development of drug resistance. Proc Natl Acad Sci U S A. 2011;108:20509–13.

199. Friebe P, Bartenschlager R. Genetic analysis of sequences in the 3' nontranslated region of hepatitis C virus that are important for RNA replication. J Virol. 2002;76:5326–38.
200. Blight KJ, Rice CM. Secondary structure determination of the conserved 98-base sequence at the 3' terminus of hepatitis C virus genome RNA. J Virol. 1997;71:7345–52.
201. Diviney S, Tuplin A, Struthers M, Armstrong V, Elliott RM, Simmonds P, Evans DJ. A hepatitis C virus cis-acting replication element forms a long-range RNA-RNA interaction with upstream RNA sequences in NS5B. J Virol. 2008;82:9008–22.
202. Friebe P, Boudet J, Simorre JP, Bartenschlager R. Kissing-loop interaction in the 3' end of the hepatitis C virus genome essential for RNA replication. J Virol. 2005;79:380–92.
203. Tuplin A, Struthers M, Simmonds P, Evans DJ. A twist in the tail: SHAPE mapping of long-range interactions and structural rearrangements of RNA elements involved in HCV replication. Nucleic Acids Res. 2012;40:6908–21.
204. Gwack Y, Kim DW, Han JH, Choe J. Characterization of RNA binding activity and RNA helicase activity of the hepatitis C virus NS3 protein. Biochem Biophys Res Commun. 1996;225:654–9.
205. Lohmann V, Korner F, Herian U, Bartenschlager R. Biochemical properties of hepatitis C virus NS5B RNA-dependent RNA polymerase and identification of amino acid sequence motifs essential for enzymatic activity. J Virol. 1997;71:8416–28.
206. You S, Rice CM. 3' RNA elements in hepatitis C virus replication: kissing partners and long poly(U). J Virol. 2008;82:184–95.
207. Jopling CL, Yi M, Lancaster AM, Lemon SM, Sarnow P. Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. Science. 2005;309:1577–81.
208. Roberts AP, Lewis AP, Jopling CL. miR-122 activates hepatitis C virus translation by a specialized mechanism requiring particular RNA components. Nucleic Acids Res. 2011;39:7716–29.
209. Lohmann V. Hepatitis C virus RNA replication. Curr Top Microbiol Immunol. 2013;369:167–98.
210. Blackham S, Baillie A, Al-Hababi F, Remlinger K, You S, Hamatake R, McGarvey MJ. Gene expression profiling indicates the roles of host oxidative stress, apoptosis, lipid metabolism, and intracellular transport genes in the replication of hepatitis C virus. J Virol. 2010;84:5404–14.
211. Diamond DL, Syder AJ, Jacobs JM, Sorensen CM, Walters KA, Proll SC, McDermott JE, Gritsenko MA, Zhang Q, Zhao R, Metz TO, Camp DG 2nd, Waters KM, Smith RD, Rice CM, Katze MG. Temporal proteome and lipidome profiles reveal hepatitis C virus-associated reprogramming of hepatocellular metabolism and bioenergetics. PLoS Pathog. 2010;6:e1000719.
212. Yamane D, McGivern DR, Wauthier E, Yi M, Madden VJ, Welsch C, Antes I, Wen Y, Chugh PE, McGee CE, Widman DG, Misumi I, Bandyopadhyay S, Kim S, Shimakami T, Oikawa T, Whitmire JK, Heise MT, Dittmer DP, Kao CC, Pitson SM, Merrill AH Jr, Reid LM, Lemon SM. Regulation of the hepatitis C virus RNA replicase by endogenous lipid peroxidation. Nat Med. 2014;20:927–35.
213. Narayanan S, Nieh AH, Kenwood BM, Davis CA, Tosello-Trampont AC, Elich TD, Breazeale SD, Ward E, Anderson RJ, Caldwell SH, Hoehn KL, Hahn YS. Distinct Roles for Intracellular and Extracellular Lipids in Hepatitis C Virus Infection. PLoS One. 2016;11:e0156996.
214. Wang C, Gale M Jr, Keller BC, Huang H, Brown MS, Goldstein JL, Ye J. Identification of FBL2 as a geranylgeranylated cellular protein required for hepatitis C virus RNA replication. Mol Cell. 2005;18:425–34.
215. Barba G, Harper F, Harada T, Kohara M, Goulinet S, Matsuura Y, Eder G, Schaff Z, Chapman MJ, Miyamura T, Brechot C. Hepatitis C virus core protein shows a cytoplasmic localization and associates to cellular lipid storage droplets. Proc Natl Acad Sci U S A. 1997;94:1200–5.
216. McLauchlan J, Lemberg MK, Hope G, Martoglio B. Intramembrane proteolysis promotes trafficking of hepatitis C virus core protein to lipid droplets. EMBO J. 2002;21:3980–8.

217. Moradpour D, Wakita T, Tokushige K, Carlson RI, Krawczynski K, Wands JR. Characterization of three novel monoclonal antibodies against hepatitis C virus core protein. J Med Virol. 1996;48:234–41.
218. Brass V, Bieck E, Montserret R, Wolk B, Hellings JA, Blum HE, Penin F, Moradpour D. An amino-terminal amphipathic alpha-helix mediates membrane association of the hepatitis C virus nonstructural protein 5A. J Biol Chem. 2002;277:8130–9.
219. Masaki T, Suzuki R, Murakami K, Aizaki H, Ishii K, Murayama A, Date T, Matsuura Y, Miyamura T, Wakita T, Suzuki T. Interaction of hepatitis C virus nonstructural protein 5A with core protein is critical for the production of infectious virus particles. J Virol. 2008;82:7964–76.
220. Targett-Adams P, Boulant S, McLauchlan J. Visualization of double-stranded RNA in cells supporting hepatitis C virus RNA replication. J Virol. 2008;82:2182–95.
221. Gao L, Aizaki H, He JW, Lai MM. Interactions between viral nonstructural proteins and host protein hVAP-33 mediate the formation of hepatitis C virus RNA replication complex on lipid raft. J Virol. 2004;78:3480–8.
222. Hamamoto I, Nishimura Y, Okamoto T, Aizaki H, Liu M, Mori Y, Abe T, Suzuki T, Lai MM, Miyamura T, Moriishi K, Matsuura Y. Human VAP-B is involved in hepatitis C virus replication through interaction with NS5A and NS5B. J Virol. 2005;79:13473–82.
223. Moriishi K, Matsuura Y. Host factors involved in the replication of hepatitis C virus. Rev Med Virol. 2007;17:343–54.
224. Evans MJ, Rice CM, Goff SP. Phosphorylation of hepatitis C virus nonstructural protein 5A modulates its protein interactions and viral RNA replication. Proc Natl Acad Sci U S A. 2004;101:13038–43.
225. Watashi K, Ishii N, Hijikata M, Inoue D, Murata T, Miyanari Y, Shimotohno K. Cyclophilin B is a functional regulator of hepatitis C virus RNA polymerase. Mol Cell. 2005;19:111–22.
226. Kaul A, Stauffer S, Berger C, Pertel T, Schmitt J, Kallis S, Zayas M, Lohmann V, Luban J, Bartenschlager R. Essential role of cyclophilin A for hepatitis C virus replication and virus production and possible link to polyprotein cleavage kinetics. PLoS Pathog. 2009;5:e1000546.
227. Liu Z, Yang F, Robotham JM, Tang H. Critical role of cyclophilin A and its prolyl-peptidyl isomerase activity in the structure and function of the hepatitis C virus replication complex. J Virol. 2009;83:6554–65.
228. Chatterji U, Bobardt M, Tai A, Wood M, Gallay PA. Cyclophilin and NS5A inhibitors, but not other anti-hepatitis C virus (HCV) agents, preclude HCV-mediated formation of double-membrane-vesicle viral factories. Antimicrob Agents Chemother. 2015;59:2496–507.
229. Shimakami T, Yamane D, Jangra RK, Kempf BJ, Spaniel C, Barton DJ, Lemon SM. Stabilization of hepatitis C virus RNA by an Ago2-miR-122 complex. Proc Natl Acad Sci U S A. 2012;109:941–6.
230. Machlin ES, Sarnow P, Sagan SM. Masking the 5' terminal nucleotides of the hepatitis C virus genome by an unconventional microRNA-target RNA complex. Proc Natl Acad Sci U S A. 2011;108:3193–8.
231. Ribeiro RM, Li H, Wang S, Stoddard MB, Learn GH, Korber BT, Bhattacharya T, Guedj J, Parrish EH, Hahn BH, Shaw GM, Perelson AS. Quantifying the diversification of hepatitis C virus (HCV) during primary infection: estimates of the in vivo mutation rate. PLoS Pathog. 2012;8:e1002881.
232. Simmonds P. Variability of hepatitis C virus. Hepatology. 1995;21:570–83.
233. Dusheiko G, Simmonds P. Sequence variability of hepatitis C virus and its clinical relevance. J Viral Hepat. 1994;1:3–15.
234. Simmonds P. The origin and evolution of hepatitis viruses in humans. J Gen Virol. 2001;82:693–712.
235. Berger KL, Cooper JD, Heaton NS, Yoon R, Oakland TE, Jordan TX, Mateu G, Grakoui A, Randall G. Roles for endocytic trafficking and phosphatidylinositol 4-kinase III alpha in hepatitis C virus replication. Proc Natl Acad Sci U S A. 2009;106:7577–82.

236. Nielsen SU, Bassendine MF, Martin C, Lowther D, Purcell PJ, King BJ, Neely D, Toms GL. Characterization of hepatitis C RNA-containing particles from human liver by density and size. J Gen Virol. 2008;89:2507–17.
237. Poenisch M, Metz P, Blankenburg H, Ruggieri A, Lee JY, Rupp D, Rebhan I, Diederich K, Kaderali L, Domingues FS, Albrecht M, Lohmann V, Erfle H, Bartenschlager R. Identification of HNRNPK as regulator of hepatitis C virus particle production. PLoS Pathog. 2015;11: e1004573.
238. Eyre NS, Fiches GN, Aloia AL, Helbig KJ, McCartney EM, McErlean CS, Li K, Aggarwal A, Turville SG, Beard MR. Dynamic imaging of the hepatitis C virus NS5A protein during a productive infection. J Virol. 2014;88:3636–52.
239. Yi Z, Fang C, Zou J, Xu J, Song W, Du X, Pan T, Lu H, Yuan Z. Affinity purification of the hepatitis C virus replicase identifies valosin-containing protein, a member of the ATPases associated with diverse cellular activities family, as an active virus replication modulator. J Virol. 2016;90:9953–66.
240. Otter-Nilsson M, Hendriks R, Pecheur-Huet EI, Hoekstra D, Nilsson T. Cytosolic ATPases, p97 and NSF, are sufficient to mediate rapid membrane fusion. EMBO J. 1999;18:2074–83.
241. Lin CC, Tsai P, Sun HY, Hsu MC, Lee JC, Wu IC, Tsao CW, Chang TT, Young KC. Apolipoprotein J, a glucose-upregulated molecular chaperone, stabilizes core and NS5A to promote infectious hepatitis C virus virion production. J Hepatol. 2014;61:984–93.
242. Huang H, Sun F, Owen DM, Li W, Chen Y, Gale M Jr, Ye J. Hepatitis C virus production by human hepatocytes dependent on assembly and secretion of very low-density lipoproteins. Proc Natl Acad Sci U S A. 2007;104:5848–53.
243. Benga WJ, Krieger SE, Dimitrova M, Zeisel MB, Parnot M, Lupberger J, Hildt E, Luo G, McLauchlan J, Baumert TF, Schuster C. Apolipoprotein E interacts with hepatitis C virus nonstructural protein 5A and determines assembly of infectious particles. Hepatology. 2010;51:43–53.
244. Cun W, Jiang J, Luo G. The C-terminal alpha-helix domain of apolipoprotein E is required for interaction with nonstructural protein 5A and assembly of hepatitis C virus. J Virol. 2010;84:11532–41.
245. Lee JY, Acosta EG, Stoeck IK, Long G, Hiet MS, Mueller B, Fackler OT, Kallis S, Bartenschlager R. Apolipoprotein E likely contributes to a maturation step of infectious hepatitis C virus particles and interacts with viral envelope glycoproteins. J Virol. 2014;88:12422–37.
246. Chang KS, Jiang J, Cai Z, Luo G. Human apolipoprotein e is required for infectivity and production of hepatitis C virus in cell culture. J Virol. 2007;81:13783–93.
247. Icard V, Diaz O, Scholtes C, Perrin-Cocon L, Ramiere C, Bartenschlager R, Penin F, Lotteau V, Andre P. Secretion of hepatitis C virus envelope glycoproteins depends on assembly of apolipoprotein B positive lipoproteins. PLoS One. 2009;4:e4233.
248. Bartenschlager R, Penin F, Lohmann V, Andre P. Assembly of infectious hepatitis C virus particles. Trends Microbiol. 2011;19:95–103.
249. Salloum S, Wang H, Ferguson C, Parton RG, Tai AW. Rab18 binds to hepatitis C virus NS5A and promotes interaction between sites of viral replication and lipid droplets. PLoS Pathog. 2013;9:e1003513.
250. Pham TM, Tran SC, Lim YS, Hwang SB. Hepatitis C virus-induced Rab32 aggregation and its implications for virion assembly. J Virol. 2017;91:e01662–16.
251. Menzel N, Fischl W, Hueging K, Bankwitz D, Frentzen A, Haid S, Gentzsch J, Kaderali L, Bartenschlager R, Pietschmann T. MAP-kinase regulated cytosolic phospholipase A2 activity is essential for production of infectious hepatitis C virus particles. PLoS Pathog. 2012;8: e1002829.
252. Cottarel J, Plissonnier ML, Kullolli M, Pitteri S, Clement S, Millarte V, Si-Ahmed SN, Farhan H, Zoulim F, Parent R. FIG 4 is a hepatitis C virus particle-bound protein implicated in virion morphogenesis and infectivity with cholesteryl ester modulation potential. J Gen Virol. 2016;97:69–81.

253. Vieyres G, Welsch K, Gerold G, Gentzsch J, Kahl S, Vondran FW, Kaderali L, Pietschmann T. ABHD5/CGI-58, the Chanarin-Dorfman Syndrome Protein, Mobilises Lipid Stores for Hepatitis C Virus Production. PLoS Pathog. 2016;12:e1005568.
254. Rosch K, Kwiatkowski M, Hofmann S, Schobel A, Gruttner C, Wurlitzer M, Schluter H, Herker E. Quantitative lipid droplet proteome analysis identifies annexin A3 as a cofactor for HCV particle production. Cell Rep. 2016;16:3219–31.
255. Cai H, Yao W, Li L, Li X, Hu L, Mai R, Peng T. Cell-death-inducing DFFA-like Effector B Contributes to the Assembly of Hepatitis C Virus (HCV) Particles and Interacts with HCV NS5A. Sci Rep. 2016;6:27778.
256. Li Q, Pene V, Krishnamurthy S, Cha H, Liang TJ. Hepatitis C virus infection activates an innate pathway involving IKK-alpha in lipogenesis and viral assembly. Nat Med. 2013;19:722–9.
257. Coller KE, Heaton NS, Berger KL, Cooper JD, Saunders JL, Randall G. Molecular determinants and dynamics of hepatitis C virus secretion. PLoS Pathog. 2012;8:e1002466.
258. Jiang J, Luo G. Apolipoprotein E but not B is required for the formation of infectious hepatitis C virus particles. J Virol. 2009;83:12680–91.
259. Gastaminza P, Cheng G, Wieland S, Zhong J, Liao W, Chisari FV. Cellular determinants of hepatitis C virus assembly, maturation, degradation, and secretion. J Virol. 2008;82:2120–9.
260. Fukuhara T, Wada M, Nakamura S, Ono C, Shiokawa M, Yamamoto S, Motomura T, Okamoto T, Okuzaki D, Yamamoto M, Saito I, Wakita T, Koike K, Matsuura Y. Amphipathic alpha-helices in apolipoproteins are crucial to the formation of infectious hepatitis C virus particles. PLoS Pathog. 2014;10:e1004534.
261. Neveu G, Barouch-Bentov R, Ziv-Av A, Gerber D, Jacob Y, Einav S. Identification and targeting of an interaction between a tyrosine motif within hepatitis C virus core protein and AP2M1 essential for viral assembly. PLoS Pathog. 2012;8:e1002845.
262. Benedicto I, Gondar V, Molina-Jimenez F, Garcia-Buey L, Lopez-Cabrera M, Gastaminza P, Majano PL. Clathrin mediates infectious hepatitis C virus particle egress. J Virol. 2015;89:4180–90.
263. Corless L, Crump CM, Griffin SD, Harris M. Vps4 and the ESCRT-III complex are required for the release of infectious hepatitis C virus particles. J Gen Virol. 2010;91:362–72.
264. Lai CK, Jeng KS, Machida K, Lai MM. Hepatitis C virus egress and release depend on endosomal trafficking of core protein. J Virol. 2010;84:11590–8.
265. Mankouri J, Walter C, Stewart H, Bentham M, Park WS, Heo WD, Fukuda M, Griffin S, Harris M. Release of infectious hepatitis C virus from Huh7 cells occurs via a trans-Golgi network-to-endosome pathway independent of very-low-density lipoprotein secretion. J Virol. 2016;90:7159–70.
266. Ploen D, Hafirassou ML, Himmelsbach K, Schille SA, Biniossek ML, Baumert TF, Schuster C, Hildt E. TIP47 is associated with the hepatitis C virus and its interaction with Rab9 is required for release of viral particles. Eur J Cell Biol. 2013;92:374–82.
267. Shrivastava S, Devhare P, Sujijantarat N, Steele R, Kwon YC, Ray R, Ray RB. Knockdown of autophagy inhibits infectious hepatitis C virus release by the exosomal pathway. J Virol. 2015;90:1387–96.
268. Frese M, Pietschmann T, Moradpour D, Haller O, Bartenschlager R. Interferon-alpha inhibits hepatitis C virus subgenomic RNA replication by an MxA-independent pathway. J Gen Virol. 2001;82:723–33.
269. Hopf U, Moller B, Konig V, Kuther S, Lobeck H, Huhn D. Long-term treatment of cryptogenic hepatitis C using recombinant interferon alpha. Z Gastroenterol. 1990;28:453–7.
270. Pawlotsky JM. Treatment failure and resistance with direct-acting antiviral drugs against hepatitis C virus. Hepatology. 2011;53:1742–51.
271. Hazuda DJ, Burroughs M, Howe AY, Wahl J, Venkatraman S. Development of boceprevir: a first-in-class direct antiviral treatment for chronic hepatitis C infection. Ann N Y Acad Sci. 2013;1291:69–76.

272. Kwong AD, Kauffman RS, Hurter P, Mueller P. Discovery and development of telaprevir: an NS3-4A protease inhibitor for treating genotype 1 chronic hepatitis C virus. Nat Biotechnol. 2011;29:993–1003.
273. Romano KP, Ali A, Aydin C, Soumana D, Ozen A, Deveau LM, Silver C, Cao H, Newton A, Petropoulos CJ, Huang W, Schiffer CA. The molecular basis of drug resistance against hepatitis C virus NS3/4A protease inhibitors. PLoS Pathog. 2012;8:e1002832.
274. Pawlotsky JM. Hepatitis C treatment: the data flood goes on-an update from the liver meeting 2014. Gastroenterology. 2015;148:468–79.
275. Wang S, Wang Y, Wang J, Sato T, Izawa K, Soloshonok VA, Liu H. The second-generation of highly potent hepatitis C virus (HCV) NS3/4A protease inhibitors: evolutionary design based on tailor-made amino acids, synthesis and major features of bio-activity. Curr Pharm Des. 2017;23:4493–554.
276. Summa V, Ludmerer SW, McAauley JA, Fandozzi C, Burlein C, Claudio G, Coleman PJ, Dimuzio JM, Ferrara M, Di Filippo M, Gates AT, Graham DJ, Harper S, Hazuda DJ, Huang Q, McHale C, Monteagudo E, Pucci V, Rowley M, Rudd MT, Soriano A, Stahlhut MW, Vacca JP, Olsen DB, Liverton NJ, Carroll SS. MK-5172, a selective inhibitor of hepatitis C virus NS3/4a protease with broad activity across genotypes and resistant variants. Antimicrob Agents Chemother. 2012;56:4161–7.
277. Burstow NJ, Mohamed Z, Gomaa AI, Sonderup MW, Cook NA, Waked I, Spearman CW, Taylor-Robinson SD. Hepatitis C treatment: where are we now? Int J Gen Med. 2017;10:39–52.
278. Deore RR, Chern JW. NS5B RNA dependent RNA polymerase inhibitors: the promising approach to treat hepatitis C virus infections. Curr Med Chem. 2010;17:3806–26.
279. Appleby TC, Perry JK, Murakami E, Barauskas O, Feng J, Cho A, Fox D 3rd, Wetmore DR, McGrath ME, Ray AS, Sofia MJ, Swaminathan S, Edwards TE. Viral replication. Structural basis for RNA replication by the hepatitis C virus polymerase. Science. 2015;347:771–5.
280. Sofia MJ, Chang W, Furman PA, Mosley RT, Ross BS. Nucleoside, nucleotide, and non-nucleoside inhibitors of hepatitis C virus NS5B RNA-dependent RNA-polymerase. J Med Chem. 2012;55:2481–531.
281. Dyson JK, Hutchinson J, Harrison L, Rotimi O, Tiniakos D, Foster GR, Aldersley MA, McPherson S. Liver toxicity associated with sofosbuvir, an NS5A inhibitor and ribavirin use. J Hepatol. 2016;64:234–8.
282. Berger C, Romero-Brey I, Radujkovic D, Terreux R, Zayas M, Paul D, Harak C, Hoppe S, Gao M, Penin F, Lohmann V, Bartenschlager R. Daclatasvir-like inhibitors of NS5A block early biogenesis of hepatitis C virus-induced membranous replication factories, independent of RNA replication. Gastroenterology. 2014;147:1094–105.e25.
283. Ascher DB, Wielens J, Nero TL, Doughty L, Morton CJ, Parker MW. Potent hepatitis C inhibitors bind directly to NS5A and reduce its affinity for RNA. Sci Rep. 2014;4:4765.
284. Gotte M, Feld JJ. Direct-acting antiviral agents for hepatitis C: structural and mechanistic insights. Nat Rev Gastroenterol Hepatol. 2016;13:338–51.
285. Krishnan P, Tripathi R, Schnell G, Reisch T, Beyer J, Irvin M, Xie W, Larsen L, Cohen D, Podsadecki T, Pilot-Matias T, Collins C. Resistance analysis of baseline and treatment-emergent variants in hepatitis C virus genotype 1 in the AVIATOR study with paritaprevir-ritonavir, ombitasvir, and dasabuvir. Antimicrob Agents Chemother. 2015;59:5445–54.
286. Forns X, Lee SS, Valdes J, Lens S, Ghalib R, Aguilar H, Felizarta F, Hassanein T, Hinrichsen H, Rincon D, Morillas R, Zeuzem S, Horsmans Y, Nelson DR, Yu Y, Krishnan P, Lin CW, Kort JJ, Mensa FJ. Glecaprevir plus pibrentasvir for chronic hepatitis C virus genotype 1, 2, 4, 5, or 6 infection in adults with compensated cirrhosis (EXPEDITION-1): a single-arm, open-label, multicentre phase 3 trial. Lancet Infect Dis. 2017;17:1062–8.
287. Meuleman P, Hesselgesser J, Paulson M, Vanwolleghem T, Desombere I, Reiser H, Leroux-Roels G. Anti-CD81 antibodies can prevent a hepatitis C virus infection in vivo. Hepatology. 2008;48:1761–8.

288. Mailly L, Xiao F, Lupberger J, Wilson GK, Aubert P, Duong FHT, Calabrese D, Leboeuf C, Fofana I, Thumann C, Bandiera S, Lutgehetmann M, Volz T, Davis C, Harris HJ, Mee CJ, Girardi E, Chane-Woon-Ming B, Ericsson M, Fletcher N, Bartenschlager R, Pessaux P, Vercauteren K, Meuleman P, Villa P, Kaderali L, Pfeffer S, Heim MH, Neunlist M, Zeisel MB, Dandri M, McKeating JA, Robinet E, Baumert TF. Clearance of persistent hepatitis C virus infection in humanized mice using a claudin-1-targeting monoclonal antibody. Nat Biotechnol. 2015;33:549–54.
289. Vercauteren K, Van Den Eede N, Mesalam AA, Belouzard S, Catanese MT, Bankwitz D, Wong-Staal F, Cortese R, Dubuisson J, Rice CM, Pietschmann T, Leroux-Roels G, Nicosia A, Meuleman P. Successful anti-scavenger receptor class B type I (SR-BI) monoclonal antibody therapy in humanized mice after challenge with HCV variants with in vitro resistance to SR-BI-targeting agents. Hepatology. 2014;60:1508–18.
290. Syder AJ, Lee H, Zeisel MB, Grove J, Soulier E, MacDonald J, Chow S, Chang J, Baumert TF, McKeating JA, McKelvy J, Wong-Staal F. Small molecule scavenger receptor BI antagonists are potent HCV entry inhibitors. J Hepatol. 2011;54:48–55.
291. Rowe IA, Tully DC, Armstrong MJ, Parker R, Guo K, Barton D, Morse GD, Venuto CS, Ogilvie CB, Hedegaard DL, McKelvy JF, Wong-Staal F, Allen TM, Balfe P, McKeating JA, Mutimer DJ. Effect of scavenger receptor class B type I antagonist ITX5061 in patients with hepatitis C virus infection undergoing liver transplantation. Liver Transpl. 2016;22:287–97.
292. Fried MW, Navarro VJ, Afdhal N, Belle SH, Wahed AS, Hawke RL, Doo E, Meyers CM, Reddy KR. Effect of silymarin (milk thistle) on liver disease in patients with chronic hepatitis C unsuccessfully treated with interferon therapy: a randomized controlled trial. JAMA. 2012;308:274–82.
293. Wagoner J, Negash A, Kane OJ, Martinez LE, Nahmias Y, Bourne N, Owen DM, Grove J, Brimacombe C, McKeating JA, Pecheur EI, Graf TN, Oberlies NH, Lohmann V, Cao F, Tavis JE, Polyak SJ. Multiple effects of silymarin on the hepatitis C virus lifecycle. Hepatology. 2010;51:1912–21.
294. Ferenci P, Scherzer TM, Kerschner H, Rutter K, Beinhardt S, Hofer H, Schoniger-Hekele M, Holzmann H, Steindl-Munda P. Silibinin is a potent antiviral agent in patients with chronic hepatitis C not responding to pegylated interferon/ribavirin therapy. Gastroenterology. 2008;135:1561–7.
295. Esser-Nobis K, Romero-Brey I, Ganten TM, Gouttenoire J, Harak C, Klein R, Schemmer P, Binder M, Schnitzler P, Moradpour D, Bartenschlager R, Polyak SJ, Stremmel W, Penin F, Eisenbach C, Lohmann V. Analysis of hepatitis C virus resistance to silibinin in vitro and in vivo points to a novel mechanism involving nonstructural protein 4B. Hepatology. 2013;57:953–63.
296. Braun DL, Rauch A, Aouri M, Durisch N, Eberhard N, Anagnostopoulos A, Ledergerber B, Mullhaupt B, Metzner KJ, Decosterd L, Boni J, Weber R, Fehr J. A lead-in with silibinin prior to triple-therapy translates into favorable treatment outcomes in difficult-to-treat HIV/hepatitis C coinfected patients. PLoS One. 2015;10:e0133028.
297. Vausselin T, Calland N, Belouzard S, Descamps V, Douam F, Helle F, Francois C, Lavillette D, Duverlie G, Wahid A, Feneant L, Cocquerel L, Guerardel Y, Wychowski C, Biot C, Dubuisson J. The antimalarial ferroquine is an inhibitor of hepatitis C virus. Hepatology. 2013;58:86–97.
298. Peymani P, Ghavami S, Yeganeh B, Tabrizi R, Sabour S, Geramizadeh B, Fattahi MR, Ahmadi SM, Lankarani KB. Effect of chloroquine on some clinical and biochemical parameters in non-response chronic hepatitis C virus infection patients: pilot clinical trial. Acta Biomed. 2016;87:46–53.
299. Jopling CL. Regulation of hepatitis C virus by microRNA-122. Biochem Soc Trans. 2008;36:1220–3.
300. Janssen HL, Kauppinen S, Hodges MR. HCV infection and miravirsen. N Engl J Med. 2013;369:878.

301. Van Der Ree MH, Van Der Meer AJ, Van Nuenen AC, De Bruijne J, Ottosen S, Janssen HL, Kootstra NA, Reesink HW. Miravirsen dosing in chronic hepatitis C patients results in decreased microRNA-122 levels without affecting other microRNAs in plasma. Aliment Pharmacol Ther. 2016;43:102–13.
302. Bartosch B. Piecing together the key players of fibrosis in chronic hepatitis C: what roles do non-hepatic liver resident cell types play? Gut. 2015;64:862–3.
303. Zeng C, Wang YL, Xie C, Sang Y, Li TJ, Zhang M, Wang R, Zhang Q, Zheng L, Zhuang SM. Identification of a novel TGF-beta-miR-122-fibronectin 1/serum response factor signaling cascade and its implication in hepatic fibrogenesis. Oncotarget. 2015;6:12224–33.
304. Chatterji U, Bobardt M, Schaffer L, Wood M, Gallay PA. Cyclophilin Inhibitors Remodel the Endoplasmic Reticulum of HCV-Infected Cells in a Unique Pattern Rendering Cells Impervious to a Reinfection. PLoS One. 2016;11:e0159511.
305. Gane E, Stedman C, Dole K, Chen J, Meyers CD, Wiedmann B, Zhang J, Raman P, Colvin RA. A diacylglycerol transferase 1 inhibitor is a potent hepatitis C antiviral in vitro but not in patients in a randomized clinical trial. ACS Infect Dis. 2017;3:144–51.
306. Testoni B, Durantel D, Lebosse F, Fresquet J, Helle F, Negro F, Donato MF, Levrero M, Zoulim F. Ribavirin restores IFNalpha responsiveness in HCV-infected livers by epigenetic remodelling at interferon stimulated genes. Gut. 2016;65:672–82.

Hepatitis C Virus Origin

2

Timokratis Karamitros, Dimitrios Paraskevis, and Gkikas Magiorkinis

Is there a zoonotic origin for HCV? If so, when did HCV "jumped" into humans? To date, the origin of HCV remains largely unknown. The viral peculiarities and the observed global epidemiological patterns of HCV suggest that the virus has been infecting human species for at least several centuries if not millennia.

2.1 The Peculiar Virology of HCV

The case of HCV as a human pathogen is a peculiar outlier for the biological patterns of known human viral pathogens: it is a persistent viral pathogen with an RNA genome. Other human RNA viruses cause acute syndromes that resolve within days either with a lethal outcome or with immune reactions eradicating the virus from the host. On the other hand, DNA viruses can persist for long periods, one of the reasons being that DNA is a much more resistant molecule than RNA either within the cell or when exposed to the environment. DNA viruses can also persist by entering a latent nonreplicating stage which allows them to evade immune responses and thus linger within the host. On the other hand, only through constant replication an RNA virus can persist, as there are no known long-term "sanctuaries" for RNA molecules in the cells. Thus, HCV needs to constantly replicate its RNA genome to induce chronic carriage; at the same time, it is constantly targeted by immune responses, as it cannot enter a latent phase. So how does HCV manage to constantly replicate and at the same time survive immune responses for years? It might be that the liver, where the

T. Karamitros
Hellenic Pasteur Institute, Athens, Greece
e-mail: tkaram@pasteur.gr

D. Paraskevis · G. Magiorkinis (✉)
Department of Hygiene Epidemiology and Medical Statistics, Medical School, National and Kapodistrian University of Athens, Athens, Greece
e-mail: dparask@med.uoa.gr; gmagi@med.uoa.gr

A. Hatzakis (ed.), *Hepatitis C: Epidemiology, Prevention and Elimination*,
https://doi.org/10.1007/978-3-030-64649-3_2

virus replicates, is a chronic RNA-virus-friendly site. Indeed, the liver as the biochemical factory is favored by a peculiar immune tolerance [1], and this is probably what HCV is exploiting. Such a delicate virus-host balance suggests, probably, that the virus has persisted for long within the human host. It also suggests that if HCV came from an animal, it was most likely replicating within the liver of the animal, too. With the emergence of high-throughput sequencing technologies since 2010, we now know more about the presence and persistence of similar viruses in animal hosts.

2.2 Nonhuman Hosts of HCV-Like Viruses

Recently, a variety of HCV-associated viruses were identified in unexpected hosts like rodents, bats, monkeys, and horses. These findings can support current research on HCV natural history, pathogenicity, and hepatitis treatment through a better understanding of the virus evolution and the interactions with its host.

Until 2011, the only known homolog of HCV was GBV-B, a virus isolated from tamarins. Originally, in 1967, a surgeon (G.B.), suffering from acute hepatitis, inoculated his serum into a tamarin, and this experimental infection developed into acute hepatitis [2]. Later, in 1995, the passaged "GB" infectious agent caused hepatitis in tamarins, resulting in the identification of two new members of the *Flaviviridae* family, the GBV-A and GBV-B [3, 4]. Although GBV-A was also found in New-World monkeys, none of these viruses could infect humans. However, their genomic sequences assisted in the isolation of one more virus, named GBV-C or hepatitis G virus (HGV), this time found in human serum samples [5]. GBV-C, also known as human pegivirus (HPgV), was not associated with viral hepatitis but was frequently found in human samples (1–4% of healthy blood donors) [6]. Together with GBV-A, they belong to the genus *Pegivirus* (persistent GB viruses) of the family *Flaviviridae*, due to their ability to persist for years without any clinical symptoms [7]. GBV-B was the original GB agent, and although it was never found in primates other than the experimentally infected tamarins, it was similar to HCV, sequence-wise [7]; thus, together with HCV, they formed the second of the four genera in the *Flaviviridae* family, the hepaciviruses [8–10]. A fourth lineage within the GBV diversity, GBV-D, has been isolated from bats [11].

During the last 5–6 years, research on HCV origin has been intensified mainly driven by the dramatic developments in high-throughput sequencing technologies. In 2011 and 2012, breakthrough discoveries of novel hepaciviruses hosted by dogs [12] and horses [13] insinuated a non-primate but zoonotic source of the human HCV epidemic. Since then, other hepacivirus hosts have been also identified, such as rodents, bats, cows, and Old-World primates [14–19]. These new species now form the non-primate hepaciviruses (NPHV). Likewise, more pegiviruses have been recently identified in bats (BPgV), rodents (RPgV), and equines (EPgV and Theiler's disease-associated virus, TDAV) [15, 17, 20, 21].

The first NPHV was found in canine respiratory samples. Due to its sequence homology to HCV, it was named canine hepacivirus (CHV). Subsequent studies

supported a rare to absent viral prevalence in dogs examined [13, 22, 23]. These observations, together with evidences of unusually high—for an RNA virus—sequence conservation between the isolated samples, led to the assumption that CHV atypically and subclinically replicates in dogs. CHV discovery however was of paramount importance, since recombinant antigens that were developed based on the described CHV sequence mediated the serological screening of a wide range of species and the isolation of the equine NPHV.

Equine NPHV remains the closest relative of HCV. It is omnipresent with ~30–40% seropositive and 3–7% RNA-positive horses worldwide [13, 24], and thus it can be considered as a universal contaminant of horse-sera-involving cell cultures. Exactly like HCV in humans, it can cause persistent infections for years, with high titers of detectable RNA in the serum and the liver biopsies of horses. Genome-wise, NPHV is similar to HCV; the ~9 KB single-stranded, positive RNA genome comprises a single ORF that encodes three structural and seven nonstructural proteins. Moreover, the 5′ UTR of the two viruses are very similar, both with a single miR-122 seed site and a very similar type IV internal ribosome entry site (IRES) [13]. NPHV has been also shown to mimic known HCV strategies like the cleavage of the mitochondrial antiviral signaling protein (MAVS) and the Toll-IL-1 receptor domain-containing adaptor-inducing interferon-beta (TRIF). These NS3/4A serine protease proteolytic activities are HCV-characteristic virus-host interactions that could be deployed by the NPHV in vivo and potentially disrupt the human innate antiviral defense signaling pathway [5], while NS3 proteases of GBV-A and GBV-C viruses are functional as well [25]. Nevertheless, these observations support the evolutionary relationship between NPHV and HCV.

Following the discovery of the first NPHV, surveys in the American, European, and African continent have been conducted in order to identify alternative hosts. Rodent hepaciviruses (RHV) have been found in several mice, rats, and bank voles (*Chaetodipus hispidus*, *Peromyscus* spp., *Myodes glareolus*, *Neotoma lepida*, *Rhabdomys pumilio*) with an estimated prevalence of 2–3% [14, 15]. The genomic arrangement of these viruses does not differ significantly from that of other hepaciviruses. Two specific RHV isolates from European bank voles (*Myodes glareolus*) provide evidence of recombination as they share IRES sequences very similar to that of pegiviruses. These events might have happened in coinfected animals [26]. Their genome is not as conserved as their human- and horse-hosted homologs. For example, strain-specific differences are observed in the sequences of 5′ and 3′ UTRs, while some predicted alternative open reading frames (ORFs) are not omnipresent across the isolates [14]. The RHV research results expand and apply largely in bat hepaciviruses (BHV) and pegiviruses (BPgV) as well. The diversity reported for both genera is also high, and they present a higher seropositivity (5–10%) compared to RHVs [14, 15, 17, 27]. They present similar genomic arrangement and several alternative ORFs in some isolates [17]. Old-World monkeys are also a pool of hepaciviruses hosts. Guereza hepacivirus (GHV) found in colobus monkeys is the first proof of a nonhuman primate infection. GHV presents a standard hepacivirus genomic organization but has an unusually variable NS5A gene, which encodes for long sequences of disordered amino acids [16].

These findings indicate the importance of understanding the biology of animal viruses, the characterization of zoonotic reservoirs, and the elucidation of their epidemiological traits. Although the interspecies transmission of viral agents is a phenomenon that is restricted by virus-host specificity, prolonged periods of contact with domestic animals can increase the probability of virus adaptation to and/or infection of a new host (human), even through intermediate hosts [28].

2.3 The Paradox of the Global HCV

HCV is transmitted mainly through blood contact (e.g., injections, tattooing, and unsafe medical practices). Sexual transmission of HCV has been reported among HIV-infected Men Having Sex with Men [29], where it is thought that immunosuppression as well as blood-contact played significant roles. How did such a difficult-to-transmit pathogen "survive" over these hypothetical centuries of human-virus coexistence? Most importantly how did such a difficult-to-transmit pathogen spread around the globe?

Clues about the paradoxical global prevalence of HCV and the mystery of sustainable transmission among human populations emerge once we disentangle the molecular signature of its spread through phylogenetic analyses. The molecular types of HCV (known as genotypes; see the molecular epidemiology chapter) can be categorized into the pandemic (or cosmopolitan) types, which are found all around the globe, and the endemic types, which are found in specific localities.

Molecular clock analyses of the most prevalent global types (genotypes 1a and 1b) showed that their global spread emerged around World War II and their number increased in two waves [30]. The global dissemination coincided with the emergence of blood transfusion and widespread use of plasma transfusion between 1940 and 1960. One theory suggests that the use of freeze-dried plasma by Allies was the major vehicle for the observed fast dissemination of subtypes 1a and 1b around the globe: firstly plasma was produced by pooling multiple donations (which increased the probability of contamination) and secondly the procedure of freeze-drying allowed the plasma to travel long distances from the point of donation while infectivity was sustained [31]. In line with this theory, molecular phylogeography placed North America at the epicenter of the pandemic which spread the virus to other developed countries and finally to the developing world (Fig. 2.1). The numbers of these subtypes increased in two waves one soon after World War II and the other between 1960s and 1970s, which coincided with the emergence of intravenous drug abuse practices (Fig. 2.2). Thus, the global dissemination of the most prevalent subtypes 1a and 1b are indeed a very recent event in the human history.

The other pandemic types 2 and 3 seem to have originated from West-Central Africa; the global dissemination of these genotypes is hypothesized to have been facilitated through the colonial international relationships and slave trade [32, 33]. Crucially, HCV strains isolated from West-Central Africa have been attributed to every clade of HCV genetic diversity apart from genotype

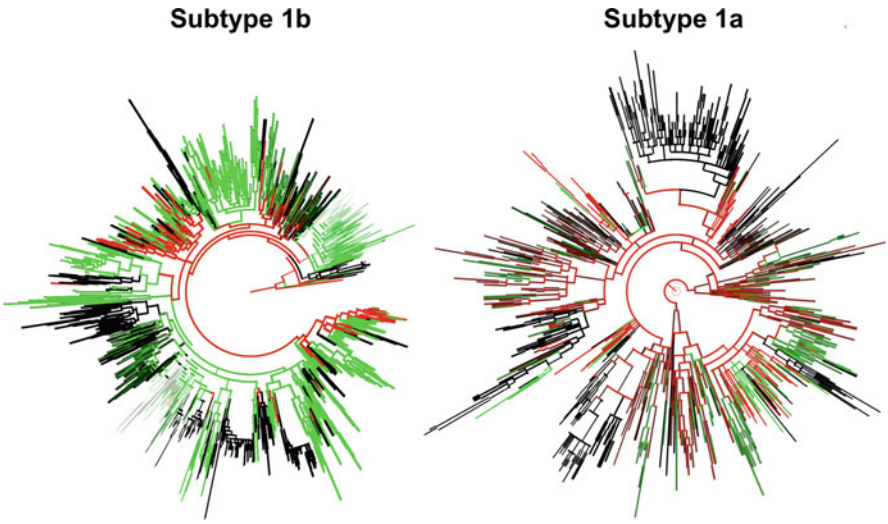


Fig. 2.1 Phylogeographic tree of HCV subtypes 1a and 1b (published in Magiorkinig et al., PLoS Medicine, 2009) depicting the most parsimonious scenario for the spread of these two subtypes between North America (red), developed countries, (green), and developing countries (black). In both trees, the root of the tree is "occupied" by the North America origin, while the developing countries occupy the tips of the tree. This suggests that the spread of these two subtypes most likely occurred from North America to the developing countries through the developed world

6 [34, 35]. The latter has been mainly isolated at Southeast Asia (for more details about the distribution of genotypes, see our molecular epidemiology chapter). We, thus, see two epicenters of HCV genetic diversity, West-Central Africa and Southeast Asia, but the connection between these two areas of genetic diversity of HCV remains unclear. Whether HCV originated from a single animal-human transmission event and subsequently somehow split between Africa and Southeast Asia or two animal-human transmission events that independently took place in Africa and Southeast Asia remains unknown.

In the absence of a potential animal reservoir for HCV, the most parsimonious scenario of the very old (centuries to millennia old) single animal-to-human transmission event remains the most plausible explanation. The alternative scenario is still likely, HCV could be the result of multiple animal-to-human transmission events, similarly to what has been observed with HIV. In order to explain the present-day global distribution of two epicenters of HCV diversity, the first scenario would be compatible with a very old transmission event, one that predates the out of Africa human migration (~50,000 years ago). In that occasion HCV could have followed humans during the out of Africa migrations, but due to low prevalence and limited transmissibility remained sustainable in specific populations (maybe only in populations that practiced rituals involving blood [36]). The second scenario can be compatible with a much more recent transmission event (at the scale of centuries ago), which occurred independently in Africa and Southeast Asia from the

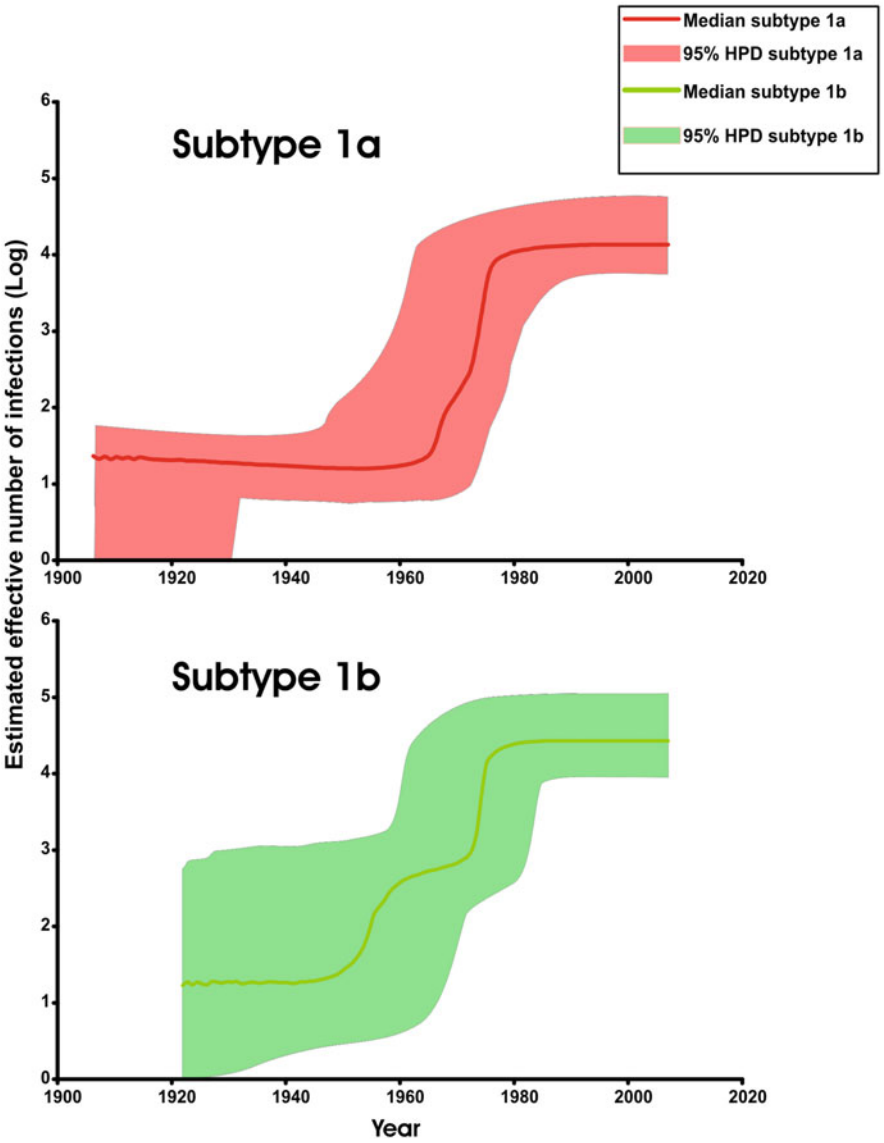


Fig. 2.2 Phylodynamic analysis of HCV subtypes 1a and 1b (published in Magiorkinig et al., PLoS Medicine, 2009) showing their effective population size (a metric similar to the number of HCV-infected people) throughout the twentieth century

hypothetical natural animal reservoirs. In the following years by using high-throughput sequencing to discover novel viruses from animals and humans could provide an answer about which of the two scenarios is more likely to have been the case.

References

1. Tiegs G, Lohse AW. Immune tolerance: what is unique about the liver. J Autoimmun. 2010;34:1–6.
2. Deinhardt F, Holmes AW, Capps RB, Popper H. Studies on the transmission of human viral hepatitis to marmoset monkeys. I. Transmission of disease, serial passages, and description of liver lesions. J Exp Med. 1967;125:673–88.
3. Simons JN, Pilot-Matias TJ, Leary TP, Dawson GJ, Desai SM, Schlauder GG, Muerhoff AS, Erker JC, Buijk SL, Chalmers ML, et al. Identification of two flavivirus-like genomes in the GB hepatitis agent. Proc Natl Acad Sci U S A. 1995;92:3401–5.
4. Muerhoff AS, Leary TP, Simons JN, Pilot-Matias TJ, Dawson GJ, Erker JC, Chalmers ML, Schlauder GG, Desai SM, Mushahwar IK. Genomic organization of GB viruses A and B: two new members of the Flaviviridae associated with GB agent hepatitis. J Virol. 1995;69:5621–30.
5. Simons JN, Leary TP, Dawson GJ, Pilot-Matias TJ, Muerhoff AS, Schlauder GG, Desai SM, Mushahwar IK. Isolation of novel virus-like sequences associated with human hepatitis. Nat Med. 1995;1:564–9.
6. Alter HJ. G-pers creepers, where'd you get those papers? A reassessment of the literature on the hepatitis G virus. Transfusion. 1997;37:569–72.
7. Stapleton JT, Foung S, Muerhoff AS, Bukh J, Simmonds P. The GB viruses: a review and proposed classification of GBV-A, GBV-C (HGV), and GBV-D in genus pegivirus within the family flaviviridae. J Gen Virol. 2011;92:233–46.
8. Bukh J, Miller RH, Purcell RH. Genetic heterogeneity of hepatitis C virus: quasispecies and genotypes. Semin Liver Dis. 1995;15:41–63.
9. Simmonds P, Holmes EC, Cha TA, Chan SW, McOmish F, Irvine B, Beall E, Yap PL, Kolberg J, Urdea MS. Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. J Gen Virol. 1993;74(Pt 11):2391–9.
10. Simmonds P, Bukh J, Combet C, Deleage G, Enomoto N, Feinstone S, Halfon P, Inchauspe G, Kuiken C, Maertens G, Mizokami M, Murphy DG, Okamoto H, Pawlotsky JM, Penin F, Sablon E, Shin IT, Stuyver LJ, Thiel HJ, Viazov S, Weiner AJ, Widell A. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatology. 2005;42:962–73.
11. Epstein JH, Quan PL, Briese T, Street C, Jabado O, Conlan S, Ali Khan S, Verdugo D, Hossain MJ, Hutchison SK, Egholm M, Luby SP, Daszak P, Lipkin WI. Identification of GBV-D, a novel GB-like flavivirus from old world frugivorous bats (Pteropus giganteus) in Bangladesh. PLoS Pathog. 2010;6:e1000972.
12. Kapoor A, Simmonds P, Gerold G, Qaisar N, Jain K, Henriquez JA, Firth C, Hirschberg DL, Rice CM, Shields S, Lipkin WI. Characterization of a canine homolog of hepatitis C virus. Proc Natl Acad Sci U S A. 2011;108:11608–13.
13. Burbelo PD, Dubovi EJ, Simmonds P, Medina JL, Henriquez JA, Mishra N, Wagner J, Tokarz R, Cullen JM, Iadarola MJ, Rice CM, Lipkin WI, Kapoor A. Serology-enabled discovery of genetically diverse hepaciviruses in a new host. J Virol. 2012;86:6171–8.
14. Drexler JF, Corman VM, Muller MA, Lukashev AN, Gmyl A, Coutard B, Adam A, Ritz D, Leijten LM, van Riel D, Kallies R, Klose SM, Gloza-Rausch F, Binger T, Annan A, Adu-Sarkodie Y, Oppong S, Bourgarel M, Rupp D, Hoffmann B, Schlegel M, Kummerer BM, Kruger DH, Schmidt-Chanasit J, Setien AA, Cottontail VM, Hemachudha T, Wacharapluesadee S, Osterrieder K, Bartenschlager R, Matthee S, Beer M, Kuiken T, Reusken C, Leroy EM, Ulrich RG, Drosten C. Evidence for novel hepaciviruses in rodents. PLoS Pathog. 2013;9:e1003438.
15. Kapoor A, Simmonds P, Scheel TK, Hjelle B, Cullen JM, Burbelo PD, Chauhan LV, Duraisamy R, Sanchez Leon M, Jain K, Vandegrift KJ, Calisher CH, Rice CM, Lipkin WI. Identification of rodent homologs of hepatitis C virus and pegiviruses. MBio. 2013;4:e00216–00213.

16. Lauck M, Sibley SD, Lara J, Purdy MA, Khudyakov Y, Hyeroba D, Tumukunde A, Weny G, Switzer WM, Chapman CA, Hughes AL, Friedrich TC, O'Connor DH, Goldberg TL. A novel hepacivirus with an unusually long and intrinsically disordered NS5A protein in a wild Old World primate. J Virol. 2013;87:8971–81.
17. Quan PL, Firth C, Conte JM, Williams SH, Zambrana-Torrelio CM, Anthony SJ, Ellison JA, Gilbert AT, Kuzmin IV, Niezgoda M, Osinubi MO, Recuenco S, Markotter W, Breiman RF, Kalemba L, Malekani J, Lindblade KA, Rostal MK, Ojeda-Flores R, Suzan G, Davis LB, Blau DM, Ogunkoya AB, Alvarez Castillo DA, Moran D, Ngam S, Akaibe D, Agwanda B, Briese T, Epstein JH, Daszak P, Rupprecht CE, Holmes EC, Lipkin WI. Bats are a major natural reservoir for hepaciviruses and pegiviruses. Proc Natl Acad Sci U S A. 2013;110:8194–9.
18. Corman VM, Grundhoff A, Baechlein C, Fischer N, Gmyl A, Wollny R, Dei D, Ritz D, Binger T, Adankwah E, Marfo KS, Annison L, Annan A, Adu-Sarkodie Y, Oppong S, Becher P, Drosten C, Drexler JF. Highly divergent hepaciviruses from African cattle. J Virol. 2015;89:5876–82.
19. Baechlein C, Fischer N, Grundhoff A, Alawi M, Indenbirken D, Postel A, Baron AL, Offinger J, Becker K, Beineke A, Rehage J, Becher P. Identification of a novel hepacivirus in domestic cattle from Germany. J Virol. 2015;89:7007–15.
20. Chandriani S, Skewes-Cox P, Zhong W, Ganem DE, Divers TJ, Van Blaricum AJ, Tennant BC, Kistler AL. Identification of a previously undescribed divergent virus from the Flaviviridae family in an outbreak of equine serum hepatitis. Proc Natl Acad Sci U S A. 2013;110:E1407–15.
21. Kapoor A, Simmonds P, Cullen JM, Scheel TK, Medina JL, Giannitti F, Nishiuchi E, Brock KV, Burbelo PD, Rice CM, Lipkin WI. Identification of a pegivirus (GB virus-like virus) that infects horses. J Virol. 2013;87:7185–90.
22. Sinéad L, Amit K, Colin S, Bradley SS, Nathan DW, Geoff C, Brendan C, Bruce CM, Peter S. Nonprimate hepaciviruses in domestic horses, United Kingdom. Emerg Infect Dis J. 2012;18:1976.
23. Lyons S, Kapoor A, Schneider BS, Wolfe ND, Culshaw G, Corcoran B, Durham AE, Burden F, McGorum BC, Simmonds P. Viraemic frequencies and seroprevalence of non-primate hepacivirus and equine pegiviruses in horses and other mammalian species. J Gen Virol. 2014;95:1701–11.
24. Pfaender S, Cavalleri JM, Walter S, Doerrbecker J, Campana B, Brown RJ, Burbelo PD, Postel A, Hahn K, Anggakusuma RN, Baumgartner W, Becher P, Heim MH, Pietschmann T, Feige K, Steinmann E. Clinical course of infection and viral tissue tropism of hepatitis C virus-like nonprimate hepaciviruses in horses. Hepatology. 2015;61:447–59.
25. Patel MR, Loo YM, Horner SM, Gale M Jr, Malik HS. Convergent evolution of escape from hepaciviral antagonism in primates. PLoS Biol. 2012;10:e1001282.
26. Galli A, Bukh J. Comparative analysis of the molecular mechanisms of recombination in hepatitis C virus. Trends Microbiol. 2014;22:354–64.
27. Firth C, Bhat M, Firth MA, Williams SH, Frye MJ, Simmonds P, Conte JM, Ng J, Garcia J, Bhuva NP, Lee B, Che X, Quan P-L, Lipkin WI. Detection of zoonotic pathogens and characterization of novel viruses carried by commensal Rattus norvegicus in New York city. MBio. 2014;5:e01933–14.
28. Parrish CR, Holmes EC, Morens DM, Park E-C, Burke DS, Calisher CH, Laughlin CA, Saif LJ, Daszak P. Cross-species virus transmission and the emergence of new epidemic diseases. Microbiol Mol Biol Rev. 2008;72:457–70.
29. Vanhommerig JW, Lambers FA, Schinkel J, Geskus RB, Arends JE, van de Laar TJ, Lauw FN, Brinkman K, Gras L, Rijnders BJ, van der Meer JT, Prins M, Group MS, van der Meer JT, Molenkamp R, Mutschelknauss M, Nobel HE, Reesink HW, Schinkel J, van der Valk M, van den Berk GE, Brinkman K, Kwa D, van der Meche N, Toonen A, Vos D, van Broekhuizen M, Lauw FN, Mulder JW, Arends JE, van Kessel A, de Kroon I, Boonstra A, van der Ende ME, Hullegie S, Rijnders BJ, van de Laar TJ, Gras L, Smit C, Lambers FA, Prins M, Vanhommerig JW, van der Veldt W. Risk factors for sexual transmission of hepatitis C virus among human

immunodeficiency virus-infected men who have sex with men: a case-control study. Open Forum Infect Dis. 2015;2:ofv115.
30. Magiorkinis G, Magiorkinis E, Paraskevis D, Ho SY, Shapiro B, Pybus OG, Allain JP, Hatzakis A. The global spread of hepatitis C virus 1a and 1b: a phylodynamic and phylogeographic analysis. PLoS Med. 2009;6:e1000198.
31. United States, Army Medical Service, Coates JB. Blood program in World War II. Washington: Office of the Surgeon General, Dept. of the Army; for sale by the Superintendent of Documents; 1964.
32. Li C, Lu L, Murphy DG, Negro F, Okamoto H. Origin of hepatitis C virus genotype 3 in Africa as estimated through an evolutionary analysis of the full-length genomes of nine subtypes, including the newly sequenced 3d and 3e. J Gen Virol. 2014;95:1677–88.
33. Markov PV, van de Laar TJ, Thomas XV, Aronson SJ, Weegink CJ, van den Berk GE, Prins M, Pybus OG, Schinkel J. Colonial history and contemporary transmission shape the genetic diversity of hepatitis C virus genotype 2 in Amsterdam. J Virol. 2012;86:7677–87.
34. Pybus OG, Barnes E, Taggart R, Lemey P, Markov PV, Rasachak B, Syhavong B, Phetsouvanah R, Sheridan I, Humphreys IS, Lu L, Newton PN, Klenerman P. Genetic history of hepatitis C virus in East Asia. J Virol. 2009;83:1071–82.
35. Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, Barnes E. Global distribution and prevalence of hepatitis C virus genotypes. Hepatology. 2015;61:77–87.
36. Atrah HI, Ala FA, Gough D. Blood exchanged in ritual ceremonies as a possible route for infection with hepatitis C virus. J Clin Pathol. 1994;47:87.

3 Classification, Genetic Diversity and Global Distribution of Hepatitis C Virus (HCV) Genotypes and Subtypes

Dimitrios Paraskevis, Evangelia Georgia Kostaki, Anna Kramvis, and Gkikas Magiorkinis

3.1 HCV Classification

Hepatitis C virus (HCV) is a positive-sense single-stranded RNA virus that belongs to the family *Flaviviridae*. HCV is characterized by extensive genetic heterogeneity and has been classified into genotypes, subtypes and recombinant forms (RFs). The genetic diversity of HCV is higher than that of the human immunodeficiency virus type 1 (HIV-1) and hepatitis B virus (HBV), because HCV has been infecting humans for a longer time period compared to HIV-1 and does not have the overlapping reading frames present in HBV, which constrain the molecular evolution of HBV [1]. HIV-1 group M is the result of a cross-species transmission event from chimpanzee that occurred approximately at the beginning of the twentieth century [2], suggesting a recent origin in humans. HBV, on the other hand, has co-expanded with modern humans for approximately 34,100 years, coinciding with the dispersal of modern non-African humans [3, 4]. Although HBV has been infecting humans for several 1000 years, the presence of overlapping reading frames in the viral genome decelerates its evolutionary rate making HBV less divergent than HCV.

Based on the consensus classification and nomenclature system proposed in 2005 [5], for a HCV sequence to be classified as a new genotype or subtype, it should fulfil the following criteria: (a) cover the complete genome and be fully coding, (b) it should form a distinct phylogenetic clade from all previously available sequences,

D. Paraskevis (✉) · E. G. Kostaki · G. Magiorkinis
Department of Hygiene, Epidemiology and Medical Statistics, Medical School, National and Kapodistrian University of Athens, Athens, Greece
e-mail: dparask@med.uoa.gr; ekostakh@med.uoa.gr; gmagi@med.uoa.gr

A. Kramvis
Hepatitis Virus Diversity Research Unit, Department of Internal Medicine, School of Clinical Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
e-mail: Anna.Kramvis@wits.ac.za

A. Hatzakis (ed.), *Hepatitis C: Epidemiology, Prevention and Elimination*,
https://doi.org/10.1007/978-3-030-64649-3_3

(c) be isolated from at least three epidemiologically unrelated individuals and (d) not be an intergenotypic or intersubtypic recombinant form (RF). Based on these criteria proposed in 2005, HCV was classified into 6 genotypes, 18 subtypes and 58 provisional subtypes pending the characterization of a full-length sequence [5]. The phylogenetic tree of HCV clades using full-length coding sequences indeed supports the hierarchy (genotypes and subtypes) by 100% bootstrap values. Subtype assignment required a sequence of nearly complete coding region length differing from previously characterized subtypes by at least 15% and at least two other sequences available in core/E1 (90% of the region spanning nucleotides 869–1292 of the H77 reference sequence with accession number AF009606) [6] and NS5B (>90% of the region spanning nucleotide positions 8276–8615 of the reference strain H77). The genotype 1a sequence H77 (accession number AF00960) is the sequence used as reference for HCV numbering [5, 6].

Revised HCV classification published in 2014 based on phylogenetic analysis of over 1300 nearly full-length (>95% of the coding region) sequences, available in the public databases in May 2013, has revealed the existence of seven major clades named genotypes 1–7 [7] (Fig. 3.1). These genotypes have been further divided into 86 subtypes (June 2017 revision), which are named with a letter after the genotype (a, b, c, d, etc.) (Fig. 3.1). For the assignment of subtypes beyond the letter "w", the recommendation is to use xa, xb, . . ., xz, followed by ya, yb, . . ., yz, and za, zb, . . ., zz, thus extending the potential number of subtypes per genotype to 101 [7]. Regarding the proportion of divergent sites (>15% different sites in core and NS5B) for subtype assignment, the updated guidelines support the use of >15% threshold over the complete coding region [8]. Analysis of pairwise genetic dissimilarity (proportion of divergent sites) between different subtypes of the same genotype revealed, with only a few exceptions, that intrasubtyping genetic dissimilarity is <13%, suggesting that the threshold of >15% can be used for the assignment of new HCV genotypes or subtypes [7].

In the more recent proposal [7], sequences clustering separately from the rest of the subtypes, within a genotype and for which full-length coding sequences are unavailable, or they have not been characterized in a sufficient number of isolates, will no longer be classified as provisional new subtypes, as had been recommended by the earlier consensus proposal [5]. Remaining provisionally assigned HCV subtypes will be maintained as reported in the literature (November 2014) [7, 9]. Moreover, the criteria for the assignment of new genotypes have been changed. The previous consensus proposal recommended that new provisional genotypes could be assigned from a complete coding sequence, but additional sequences in partial genomic regions are needed to confirm the assignment of the new clade. Since 2005, only one new provisional genotype (7a) has been assigned, and thus due to the rarity of new genotypes, the revised assignment criteria suggest that only one complete coding sequence is needed for the assignment of new genotypes [7]. Thus, QC69 (EF108306) is confirmed as genotype 7a [7].

Updated lists of HCV genotypes and subtypes including alignments of representative complete HCV coding sequences are available on-line on a website hosted by the International Committee on Taxonomy of Viruses (ICTV) [9]. An updated list of

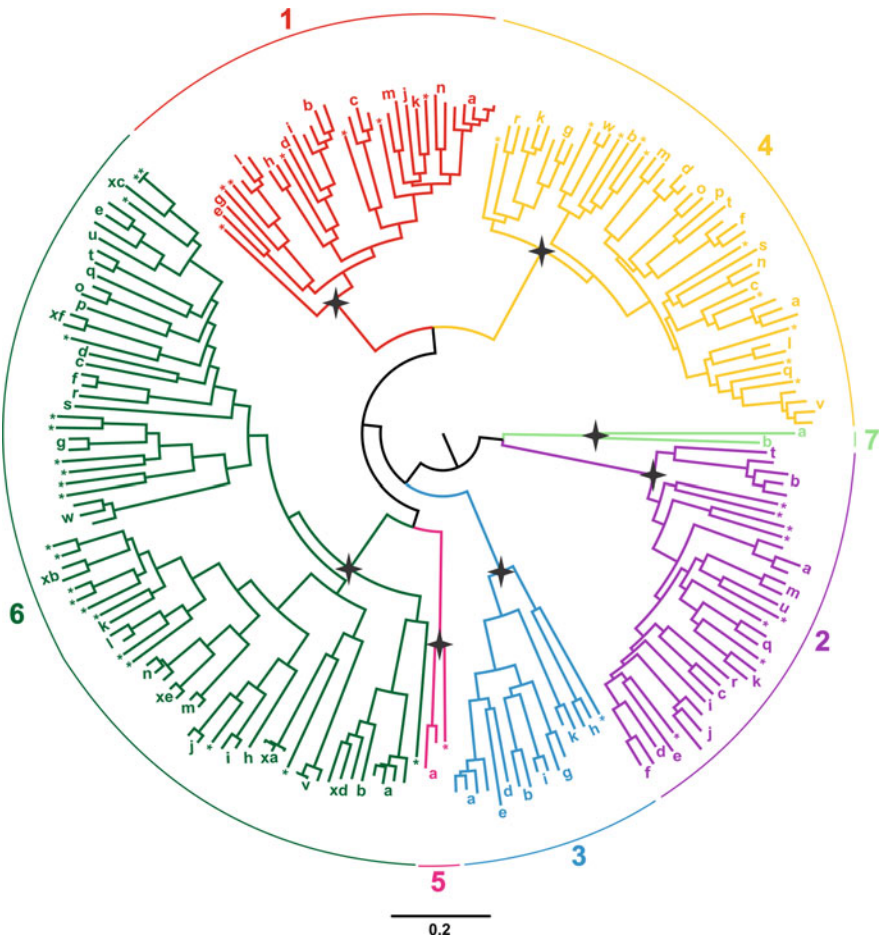


Fig. 3.1 Phylogeny of HCV major genotypes (1–7) and subtypes. Genotypes are shown in different colours. The names of genotypes are shown on the top of the corresponding clades. Stars at the nodes indicate Shimodaira-Hasegawa support equal to 1. The phylogenetic tree was inferred with approximate maximum likelihood method as implemented in FastTree program using the GTR + cat as nucleotide substitution model. Analysis was performed using representative full-length HCV genomic sequences available on a website hosted by the International Committee on Taxonomy of Viruses (ICTV)

the representative sequences for the different clades is also available on-line [9]. Previous provisional new clades (subtypes) awaiting confirmation are listed in Table 3 in the ICTV HCV website [9]. In addition, the following website (www.ictv.global/report/flaviviridae) [8] is intended to maintain the functions previously available on databases currently not maintained, namely, the HCV Los Alamos sequence database [10, 11], euHCVdb [12] and Hepatitis Virus Database (http://s2as02.genes.nig.ac.jp).

3.1.1 Recombinants

HCV intergenotypic or intersubtypic recombinants are named after recombinant forms "RF" followed by the names of the different genotypes or subtypes for which phylogenetic relationships have been documented in partial genomic regions, in the order in which they occur, separated by "/". For example, RF2k/1b, which corresponds to the intergenotypic RF comprised of genotypes 2k and 1b, was initially characterized in St. Petersburg, Russia, and has spread widely in Eurasia [13]. To date, nine different RFs have been described on complete coding region (Table 3.1). Notably, the breakpoints for all RFs are in the same genomic region (Table 3.1) [9]. Further, it is recommended that RFs, consisting of the same genotypes/subtypes comprising distinct recombination pattern(s) (different breakpoints or distinct origin), should be numbered consecutively with a numerical suffix (e.g. RF2k/1b_2).

3.1.2 Additional Hierarchy Within HCV Genotypes

Analysis of the distribution of intragenotypic pairwise p-distances (uncorrected genetic distance) revealed major differences between the six HCV genotypes. For genotypes 2, 3 and 6, there were three distributions of distances (e.g. for genotype 6, the three distributions were approximately 15–20%, 20–25% and 25–30%) versus the uniform distributions (17.7–25.4%) and (25.3–23.2%) observed for genotypes 1 and 4, respectively [7]. These observations suggest that within genotypes 2, 3 and 6, there is additional hierarchy, besides the existing subtypes, or that some of the subtypes are more closely related than others. For example, subtypes 6m and 6n or 6i, 6j and 6 h form a higher-order clade (cluster) close to the root of genotype 6 (Fig. 3.1). The different levels of clustering are reflected by the different distributions of p-distance. However, this higher level of grouping does not

Table 3.1 Recombinant (RF) HCV genomes

RF[a]	Breakpoint[b]	Accession	Isolates[c]	References
RF2k/1b	3186	AY587845	33	[13, 14]
RF2i/6p	3405–3464	DQ155560	1	[15]
RF2b/1b_1	3456	DQ364460	1	[16]
RF2/5	3366–3389	AM408911	1	[17]
RF2b/6w	3429	EU643835	1	[18]
RF2b/1b_2	3432	AB622121	1	[19]
RF2b/1a	3429–3440	JF779679	1	[20]
RF2b/1b_3	3286–3293	AB677530	1	[21]
RF2b/1b_4	3286–3293	AB677527	1	[21]

[a]Recombinant forms (RF) for which complete genome sequences are available are named according to the subtypes from which they are derived and in the order in which these appear in the genome
[b]Breakpoints are numbered with reference to H77 (AF009606)
[c]Number of individuals from whom the RF has been isolated

correspond to a common geographic origin of subtypes belonging to these clusters. Similarly, there are no distinct virological or clinical characteristics distinguishing these groups. For these reasons, no classification was decided for these higher-order groupings within genotypes 2, 3 and 6 [7].

3.2 The Global Distribution of HCV Clades

3.2.1 Introduction

The genotypes, subtypes and RFs of HCV are unequally distributed across the globe. Subtypes 1a, 1b, 2a and 3a are the most widely spread, being highly prevalent in high-income countries. They are known as the "epidemic subtypes" [22], and they have spread mostly as a result of blood and blood product transfusions, iatrogenic procedures and injecting drug use [23–25]. Their global dissemination occurred before the identification of HCV in 1989 [26]. The remaining HCV subtypes are mostly prevalent in restricted geographic areas, and they are characterized as "endemic". Genotype 1 has a global prevalence; genotypes 2 and 3 circulate mostly in West Africa and in South Asia, respectively, genotype 4 in Central Africa and genotypes 5 and 6 in Southern Africa and South East Asia, respectively [23, 27, 28]. The global distribution of the HCV clades is believed to have been shaped by historical and more recent human migration and by human practices such as transfusions and injecting drug use that started in the previous century [22].

3.2.2 Global Prevalence of HCV Clades

The global distribution of HCV clades has been estimated in two independent systematic reviews performed by Messina et al. [22] and the Polaris Observatory HCV collaborators [29]. The data presented in both studies were collected through a systematic literature search, and the estimation of the prevalence of HCV clades was based on the combination of the genotype frequencies with the Global Burden of Disease (GBD) [22], as defined by the WHO [30]. The Polaris collaboration reported a combined analysis of literature review with interviews from country experts, who provided missing data and feedback and modelling to estimate HCV prevalence on viraemic populations and distribution of HCV genotypes and subtypes [29]. Both studies provide data for a large number of countries, 117 [22] and 113 [29], accounting approximately for 90% of the global population.

The most prevalent HCV clade was genotype 1 accounting for 83.4 million cases (46.2%), with approximately one third of these infections occurring in East Asia. The second most common genotype is 3, with 54.3 million (30.1%) infections and the highest prevalence found in South Asia. Genotypes 2, 4 and 6 were estimated to have infected 16.5 million (9.1%), 15.0 million (8.3%) and 9.8 million (5.4%) individuals, respectively. Genotypes 2 and 6 dominate in East Asia and genotype 4 circulates at high prevalence in North Africa/Middle East. Genotype 5 is the least

frequent with approximately 1.4 million (<1%) cases, which mostly reside in Southern and Eastern sub-Saharan Africa [22].

3.2.3 Global Distribution of HCV Clades

The distribution of the different subtypes has been estimated by GBD region in both studies [22, 29]. Genotype 1 is more frequent across America and the Caribbean; Central Western and Eastern Europe; Central, East and Southeast Asia; Asia Pacific high-income; Australasia; and west sub-Saharan Africa. Notably, gross differences are found with regard to the proportion of subtypes 1a and 1b across the areas of high prevalence for genotype 1 (Fig. 3.2a–c). Subtype 1b is found almost exclusively among genotype 1 infections in high-income, Central and East Asia and Central and Eastern Europe, while it dominates in Western Europe and Central and Southern Latin America (Fig. 3.2c). Subtype 1a, on the other hand, has a distinct pattern, without areas of major dominance over 1b (Fig. 3.2b). Andean Latin America is the area with higher proportion of 1a transmissions than 1b, followed by North America high-income and the Caribbean. Notably, subtypes other than 1a and 1b circulate in west sub-Saharan Africa. The absence of these subtypes from sub-Saharan Africa is probably due to the fact that the dispersal of 1a and 1b is associated with blood and blood product transfusions and injections, which occurred after the Second World War from developed countries to developing world [24]. As estimated previously, the exponential phase in the subtype 1b expansion preceded that of 1a by approximately 16 years [24], thus providing a plausible explanation about the existence of areas where 1b is almost exclusively found among genotype 1 transmissions. Subtype 1b was probably introduced prior to 1a, due to blood and blood transfusions, and it has remained the most frequently circulating clade up to the present.

The most common genotypes per country have been estimated by Messina et al. [22], where genotype 1 is the most frequent clade in 73% of the countries included in the analysis. Notably in the 53% of the studies on genotype 1, for which subtypes were available, the 99% were due to subtypes 1a and 1b (31% and 68%, respectively) [22]. These findings together with the fact that 1a and 1b were introduced and spread in different areas mostly as a result of blood and blood product transfusions, iatrogenic procedures and injecting drug use [24] highlight the major impact of these newly introduced practices during the last century, in the global dissemination mainly of subtypes 1a and 1b and other clades of HCV.

Different temporal and spatial patterns have been demonstrated for the different clades of HCV mainly as a result of the abovementioned practices. Using coalescent analysis, it has been shown that a transition from constant size to rapid exponential growth (spread time) occurred at different times in the distinct geographic regions, where different genotypes/subtypes of HCV circulate. The earliest spread time was documented in Japan with subtype 1b being introduced in the 1920s as a result of schistosomiasis treatment. The spread of 1b in Spain occurred during and after the Spanish Civil War in the 1940s as a result of extensive needle sharing for penicillin treatment. Intravenous drug use has been implicated in the transmission of subtypes

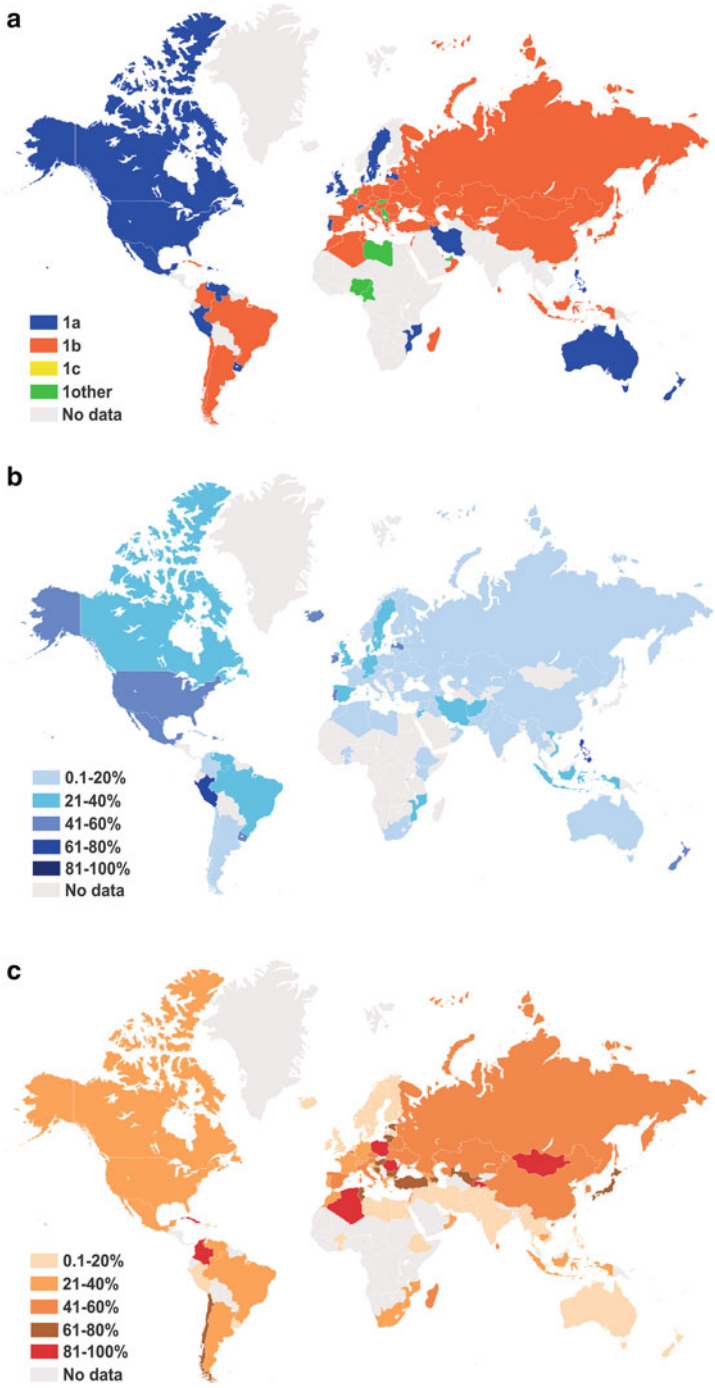


Fig. 3.2 Global distribution of HCV: (**a**) genotype 1, (**b**) subtype 1a and (**c**) subtype 1b. Colour grade reflects different proportion of HCV genotypes and subtypes

1a and 3a in the USA and the former Soviet Union, respectively, in the 1960s. Unsafe medical procedures have been implicated for the introduction of subtype 5a in South Africa in the 1950s, whereas intravenous drug abuse and blood transfusions in young patients with thalassemia were responsible for the introduction, in the 1970s, of HCV 6 in Hong Kong [31].

Another interesting metric of the global HCV genetic diversity is the Shannon diversity index as previously described [22]. The Shannon diversity index is a measure of the number of different types (such as clades) there is in a dataset and also takes into account how evenly the basic entities (such as individuals) are distributed among those types. Low values suggest that most infections are due to either 1 or 2 genotypes, while high scores indicate a more complex epidemic consisting of several genotypes. The lowest complexity is mapped in Eastern and Central sub-Saharan Africa, in North Africa/Middle East and in several countries in Central and Eastern Europe, Central Asia and Andean Latin America. On the other hand, in China, Western Europe, Southeast Asia and Australia, the complexity of the epidemic is the highest. The low score probably suggests geographic areas where the epidemic spread occurred mostly due to a single transmission route as, for example, in Eastern and Central sub-Saharan Africa, the area of dominance of genotype 4.

3.2.4 Genotype 1

The proportion of infections due to genotype 1 is the highest (>75%) in the Americas and specifically in high-income North America (USA and Canada), the Caribbean (Cuba, Martinique), Southern Central (Colombia), Andean (Bolivia, Peru) and Southern (Argentina, Chile, Uruguay) Latin America; in Western (Austria, Cyprus), Central (Albania, Bulgaria, Czech Republic, FYROM, Hungary, Poland, Romania) and Eastern (Belarus, Latvia, Moldova, Ukraine) Europe; in North Africa/Middle East (Algeria, Turkey) and Central (Azerbaijan, Mongolia, Tajikistan, Turkmenistan) and high-income (Japan, Singapore) Asia; and in West (Nigeria) and Central (Equatorial Guinea) sub-Saharan Africa (Fig. 3.2a). The areas with the lowest proportions of genotype 1 were mostly in Africa and specifically in North Africa/Middle East (Egypt, Qatar); Central (Democratic Republic of the Congo, Gabon), East (Eritrea, Ethiopia, Sudan) and West (Gambia, Guinea-Bissau) sub-Saharan Africa; and in a few countries in Asia (Laos) and the Caribbean (Suriname) (Fig. 3.2a). The areas with the lowest proportion for genotype 1 are mostly in sub-Saharan Africa probably due to the limited practice of blood and blood product transfusions and injecting drug use that were more common in developed and developing areas (Fig. 3.2a).

The relative frequencies of subtypes 1a and 1b differ greatly across the globe. Specifically, 1a dominates in Western (Great Britain, Iceland, Ireland, Netherlands, Norway, Portugal and Sweden), Central (FYROM) and Eastern (Belarus) Europe (Fig. 3.2b, c). Similarly, 1a is more frequent than 1b in North Africa/Middle East (Jordan, Iran and the UAE); in Central (Azerbaijan), South (Nepal) and Southeast (Philippines, Vietnam) Asia; in Australasia (Australia, New Zealand); in high-

income North America (Canada, USA); and in Tropical (Brazil) and Andean (Peru) Latin America [22] (Fig. 3.2b, c). The previous pattern shows that subtype 1b is more frequent in most of the areas of the developed and developing world, where the initial introduction of HCV through blood and blood product transfusions probably played a major role for the dissemination of genotype 1. The proportion of transmissions due to 1a and 1b across the globe are shown in detail in Fig. 3.2b, c.

3.2.5 Genotype 2

Subtype 2a is widely spread and belongs among the "epidemic subtypes". The proportion of genotype 2 is the highest (>50%) in West Sub-Saharan Africa (Benin, Burkina Faso, Cote d'Ivoire, Gambia, Ghana, Guinea, Guinea-Bissau) and in the Caribbean (Suriname). High proportions (25–50%) for genotype 2 were reported for North Africa/Middle East (Bahrain, Tunisia), West (Cameroon) and East (Eritrea, Ethiopia, Madagascar) sub-Saharan Africa; high-income (Republic of Korea) and Southeast (Philippines, Sri Lanka) Asia; and Central Latin America (Venezuela). This pattern suggests that the putative origin of genotype 2 is in West sub-Saharan Africa from where it probably spread to a few areas in Africa and the rest of the world. Subtypes 2a and 2b have been disseminated in developed and developing countries mostly through blood and blood product transfusions [25].

3.2.6 Genotype 3

Genotype 3 dominates in Asia and is more frequently (>50%) found in South (Afghanistan, India, Pakistan), high-income (Brunei Darussalam) and Southeast (Myanmar) Asia and in Central Europe (Slovakia). Moreover, genotype 3 circulates at proportions between 25% and 50% in several countries in Europe located in Western and Southern (Denmark, Finland, Greece, Iceland, Ireland, Luxembourg, Norway, Portugal, Sweden, Switzerland, UK), Central (Croatia, Montenegro, Slovenia) and Eastern (Russian Federation) Europe; in North Africa/Middle East (Iran, Lebanon, UAE); in Central (Georgia, Uzbekistan), South (Nepal) and Southeast (Thailand) Asia; in Australasia (Australia, New Zealand); and in tropical Latin America (Brazil) [22]. On the other hand, genotype 3 has very low frequency in sub-Saharan Africa. This pattern of dispersal suggests that the putative origin of genotype 3 is in South Asia [25, 32] and specifically in the Golden Crescent, which is one of Asia's two main opium-producing areas. Genotype 3 and especially subtype 3a have a global spread ("epidemic subtypes"), and it has high prevalence among injecting drug users [32], a pattern that is consistent with an East Asian origin in the main opium-producing areas. The low proportion of genotype 3 in sub-Saharan Africa is because the most common route for the spread of genotype 3 is injecting drug use, a practice that is not very common in sub-Saharan Africa.

3.2.7 Genotype 4

The highest proportion of genotype 4 is found mostly in sub-Saharan Africa and the Middle East. Specifically genotype 4 circulates at higher proportions (>75%) in Central (Central African Republic, Congo, Democratic Republic of the Congo, Gabon) and East sub-Saharan Africa (Sudan, Tanzania) and in North Africa/Middle East (Egypt, Qatar) [22]. Proportions for genotype 4 between 25% and 75% were found in North Africa/Middle East (Iraq, Jordan, Kuwait, Lebanon, Libya, Saudi Arabia, Syria, Tunisia) and in West (Cameroon) and East (Eritrea, Ethiopia, Kenya, Uganda) sub-Saharan Africa. Notably, genotype 4 circulates at detectable proportions (10–25%) in a few countries in Europe, such as in Cyprus, Greece, the Netherlands and Portugal, probably as a result of human mobility during the last century from Africa and the Middle East to Europe. The areas with the higher proportions of infections due to genotype 4 are restricted mostly in North Africa/Middle East and in Central East sub-Saharan Africa suggesting that the putative origin of this clade is located in this area. Subtype 4a is associated with the high HCV prevalence in Egypt [22, 29]. An epidemiologic survey in 2008 showed that blood transfusions, and parenteral anti-schistosomiasis treatment (PAT), were associated with HCV infection in Egypt [33, 34]. A large number of people (~two million) received intravenous injections as part of the anti-schistosomiasis campaign during 1964–1982 in Egypt [35, 36]. Blood transfusions, contaminated syringes and medical procedures also contributed to the dissemination of HCV in Egypt [37–41]. A molecular epidemiological study of subtype 4a strains in Greece revealed multiple introductions [42], probably as a result of population mobility of Greek populations living in Egypt for a long time, who returned to Greece during the last century (unpublished data).

3.2.8 Genotype 5

Genotype 5 has distinct characteristics since it is confined in Southern sub-Saharan Africa (Mozambique, Namibia, South Africa). Genotype 5 has also been detected at proportions (10–25%) in East sub-Saharan Africa (Eritrea, Ethiopia) and at low frequency (1–10%) in a few areas in Western Europe (Belgium, Cyprus, France), in high-income North America (Canada), in Southern Latin America (Uruguay) and in North Africa/Middle East (Syria). The high frequency of this clade in Southern sub-Saharan Africa renders this area as the most putative origin of this genotype. Molecular clock analysis of subtype 5a sequences circulating in a small area in Central France revealed that the time of the most recent common ancestor (t_{MRCA}) of the HCV 5a epidemic in France was approximately in 1939 and in Central France in 1954, estimates which were in accordance with epidemiological data [43]. HCV-5a infections in France were mostly associated with transfusion and iatrogenic and intra-familial transmissions [43].

3.2.9 Genotype 6

Genotype 6 is found mostly (>25%) in Southeast Asia (Cambodia, Vietnam, Laos). Similarly, it circulates at proportions between 10 and 25% in East (China) and Southeast (Myanmar, Thailand) Asia, while it is found in <1% in the rest of the geographic areas. Genotype 6 has the largest genetic complexity comprised of a large number of subtypes (Fig. 3.1). Notably, most of the subtypes dominate in single or neighbouring countries (6d from Vietnam, 6q from Cambodia, 6a from China/Vietnam, 6n from Thailand/Myanmar) [28]. Molecular clock and phylogeographic analysis revealed that it has originated in Asia >1000 years ago and that there are two distinct phases in its epidemic history before and during the twentieth century [28]. Genotype 6 has unique features where different subtypes spread locally during the twentieth century, and in contrast to the "epidemic subtypes", this genotype is not associated with cross-border transmissions to other geographical areas [44].

3.2.10 Genotype 7

Two full-length coding sequences have been characterized of genotype 7. Both sequences were identified in the Democratic Republic of Congo [45, 46] The first sequence (QC69) was assigned as 7a and the isolate, BAK1, was reported to belong to a HCV-7b subtype [46].

3.3 Conclusions

HCV has been characterized by extensive genetic diversity that is divided into major and secondary clades named genotypes and subtypes, respectively [7]. The classification criteria for HCV, first proposed in 2005 [1], were updated in 2014 [3], and the ICTV is maintaining websites, which provide up-to-date and current information on the genotypes/subtypes and RFs. Currently, there are 7 genotypes and 86 subtypes, which have been recognized (ICTV, June 2017 revision), and these vary in their geographic distribution globally. A similar hierarchy of classification exists for HBV (genotypes, subgenotypes) [47–50], but to a lesser extent for HIV (group M) for which a limited number of subgenotypes have been proposed [51]. HCV genotypes show higher genetic divergence than HBV and HIV and a lower prevalence and complexity of RFs [7]. The different levels of classification hierarchy reflect that the epidemic growth has followed distinct phases. It is believed that the major exponential increase in HCV transmissions occurred worldwide during the twentieth century via multiple transmission routes, including transfusions, injection drug use and unsafe medical injections [52]. During this phase, most of the HCV subtypes were generated as a result of transmission of founder strains from geographic areas with endemic infections. For example, subtypes 1b and 1a were generated as the result of multiple transmissions, through blood transfusions and injecting drug use,

respectively, of two founder strains from Africa to the rest of the world [25]. Similarly subtype 3a originated from East Asia and was dispersed worldwide through injecting drug use in the previous century. On the other hand, the emergence of the major HCV clades (genotypes) occurred probably at least >1000 years ago as suggested by a molecular epidemiology study of genotype 6 [28]. The exact chronological origin of HCV has not been inferred, but it is believed that HCV has been infecting humans for many years. The distribution of the different HCV clades differs greatly with genotypes 5 and 6, being confined to specific geographic areas, Africa and Asia, respectively. Genotype 1, on the other hand, is the most prevalent clade with subtypes 1a and 1b being the most frequent "epidemic subtypes". Similarly, 2a, 3a and 4a have been widely disseminated as "epidemic subtypes". The global distribution of the HCV genetic diversity provides an example of a pandemic that has been shaped in the last century mostly due to human practices related to transfusions, iatrogenic procedures, injecting drug use and unsafe medical injections. The study of the HCV molecular epidemiology is useful in unravelling the dispersal routes of the pathogen and also the human mobility associated with HCV infections.

Given the different transmission routes of HCV as compared to HIV and HBV, the HCV pandemic has distinct spatiotemporal characteristics. HIV has been introduced recently in humans [53, 54], and a single clade (subtype B) dominates in the high-income countries and is absent in Africa [55–57]. On the other hand, multiple non-B subtypes are frequent in Africa and in some other geographic areas in the form of epidemic infections, such as the subtype A in Russia and Eastern Europe, or in the form of sporadic infections [56, 58]. HBV subgenotype A2 and genotype D have global dissemination, but the rest of genotypes and certain subgenotypes are mostly confined to specific geographic areas [3]. HCV clades, with the exception of genotypes 5 and 6, have spread widely compared to HIV and HBV clades probably due to the different transmission routes. Parenteral transmission via contaminated blood and blood product transfusions is the major factor associated with the global expansion of the numerous HCV clades. Recent epidemic history has shaped the picture of the spatial distribution of HCV genetic heterogeneity.

References

1. Mizokami M, Orito E, Ohba K, Ikeo K, Lau JY, Gojobori T. Constrained evolution with respect to gene overlap of hepatitis B virus. J Mol Evol. 1997;44(Suppl 1):S83–90.
2. Korber B, Muldoon M, Theiler J, Gao F, Gupta R, Lapedes A, et al. Timing the ancestor of the HIV-1 pandemic strains. Science. 2000;288(5472):1789–96.
3. Paraskevis D, Magiorkinis G, Magiorkinis E, Ho SY, Belshaw R, Allain JP, et al. Dating the origin and dispersal of hepatitis B virus infection in humans and primates. Hepatology. 2013;57(3):908–16.
4. Paraskevis D, Angelis K, Magiorkinis G, Kostaki E, Ho SY, Hatzakis A. Dating the origin of hepatitis B virus reveals higher substitution rate and adaptation on the branch leading to F/H genotypes. Mol Phylogenet Evol. 2015;93:44–54.

5. Simmonds P, Bukh J, Combet C, Deleage G, Enomoto N, Feinstone S, et al. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatology. 2005;42(4):962–73.
6. Kuiken C, Combet C, Bukh J, Shin IT, Deleage G, Mizokami M, et al. A comprehensive system for consistent numbering of HCV sequences, proteins and epitopes. Hepatology. 2006;44 (5):1355–61.
7. Smith DB, Bukh J, Kuiken C, Muerhoff AS, Rice CM, Stapleton JT, et al. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource. Hepatology. 2014;59(1):318–27.
8. Simmonds P, Becher P, Bukh J, Gould EA, Meyers G, Monath T, et al. ICTV virus taxonomy profile: flaviviridae. J Gen Virol. 2017;98(1):2–3.
9. https://talk.ictvonline.org/ictv_wikis/flaviviridae/w/sg_flavi/37/table-3-remaining-provisionally-assigned-hcv-subtypes-november-2014.
10. Kuiken C, Hraber P, Thurmond J, Yusim K. The hepatitis C sequence database in Los Alamos. Nucleic Acids Res. 2008;36(Database issue):D512–6.
11. Kuiken C, Yusim K, Boykin L, Richardson R. The Los Alamos hepatitis C sequence database. Bioinformatics. 2005;21(3):379–84.
12. Combet C, Penin F, Geourjon C, Deleage G. HCVDB: hepatitis C virus sequences database. Appl Bioinforma. 2004;3(4):237–40.
13. Kalinina O, Norder H, Mukomolov S, Magnius LO. A natural intergenotypic recombinant of hepatitis C virus identified in St. Petersburg J Virol. 2002;76(8):4034–43.
14. Kalinina O, Norder H, Magnius LO. Full-length open reading frame of a recombinant hepatitis C virus strain from St Petersburg: proposed mechanism for its formation. J Gen Virol. 2004;85 (Pt 7):1853–7.
15. Noppornpanth S, Lien TX, Poovorawan Y, Smits SL, Osterhaus AD, Haagmans BL. Identification of a naturally occurring recombinant genotype 2/6 hepatitis C virus. J Virol. 2006;80(15):7569–77.
16. Kageyama S, Agdamag DM, Alesna ET, Leano PS, Heredia AM, Abellanosa-Tac-An IP, et al. A natural inter-genotypic (2b/1b) recombinant of hepatitis C virus in the Philippines. J Med Virol. 2006;78(11):1423–8.
17. Legrand-Abravanel F, Claudinon J, Nicot F, Dubois M, Chapuy-Regaud S, Sandres-Saune K, et al. New natural intergenotypic (2/5) recombinant of hepatitis C virus. J Virol. 2007;81 (8):4357–62.
18. Lee YM, Lin HJ, Chen YJ, Lee CM, Wang SF, Chang KY, et al. Molecular epidemiology of HCV genotypes among injection drug users in Taiwan: Full-length sequences of two new subtype 6w strains and a recombinant form_2b6w. J Med Virol. 2010;82(1):57–68.
19. Yokoyama K, Takahashi M, Nishizawa T, Nagashima S, Jirintai S, Yotsumoto S, et al. Identification and characterization of a natural inter-genotypic (2b/1b) recombinant hepatitis C virus in Japan. Arch Virol. 2011;156(9):1591–601.
20. Bhattacharya D, Accola MA, Ansari IH, Striker R, Rehrauer WM. Naturally occurring genotype 2b/1a hepatitis C virus in the United States. Virol J. 2011;8:458.
21. Hoshino H, Hino K, Miyakawa H, Takahashi K, Akbar SM, Mishiro S. Inter-genotypic recombinant hepatitis C virus strains in Japan noted by discrepancies between immunoassay and sequencing. J Med Virol. 2012;84(7):1018–24.
22. Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, et al. Global distribution and prevalence of hepatitis C virus genotypes. Hepatology. 2015;61(1):77–87.
23. Smith DB, Pathirana S, Davidson F, Lawlor E, Power J, Yap PL, et al. The origin of hepatitis C virus genotypes. J Gen Virol. 1997;78(Pt 2):321–8.
24. Magiorkinis G, Magiorkinis E, Paraskevis D, Ho SY, Shapiro B, Pybus OG, et al. The global spread of hepatitis C virus 1a and 1b: a phylodynamic and phylogeographic analysis. PLoS Med. 2009;6(12):e1000198.
25. Pybus OG, Cochrane A, Holmes EC, Simmonds P. The hepatitis C virus epidemic among injecting drug users. Infect Genet Evol. 2005;5(2):131–9.

26. Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science. 1989;244 (4902):359–62.
27. Simmonds P. The origin and evolution of hepatitis viruses in humans. J Gen Virol. 2001;82 (Pt 4):693–712.
28. Pybus OG, Barnes E, Taggart R, Lemey P, Markov PV, Rasachak B, et al. Genetic history of hepatitis C virus in East Asia. J Virol. 2009;83(2):1071–82.
29. Polaris Observatory HCV Collaborators. Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modelling study. Lancet Gastroenterol Hepatol. 2017;2 (3):161–76.
30. Murray CJ, Ezzati M, Flaxman AD, Lim S, Lozano R, Michaud C, et al. GBD 2010: design, definitions, and metrics. Lancet. 2012;380(9859):2063–6.
31. Tanaka Y, Kurbanov F, Mano S, Orito E, Vargas V, Esteban JI, et al. Molecular tracing of the global hepatitis C virus epidemic predicts regional patterns of hepatocellular carcinoma mortality. Gastroenterology. 2006;130(3):703–14.
32. Morice Y, Cantaloube JF, Beaucourt S, Barbotte L, De Gendt S, Goncales FL, et al. Molecular epidemiology of hepatitis C virus subtype 3a in injecting drug users. J Med Virol. 2006;78 (10):1296–303.
33. Guerra J, Garenne M, Mohamed MK, Fontanet A. HCV burden of infection in Egypt: results from a nationwide survey. J Viral Hepat. 2012;19(8):560–7.
34. Abdel-Ghaffar TY, Sira MM, El Naghi S. Hepatitis C genotype 4: the past, present, and future. World J Hepatol. 2015;7(28):2792–810.
35. Frank C, Mohamed MK, Strickland GT, Lavanchy D, Arthur RR, Magder LS, et al. The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. Lancet. 2000;355 (9207):887–91.
36. Rao MR, Naficy AB, Darwish MA, Darwish NM, Schisterman E, Clemens JD, et al. Further evidence for association of hepatitis C infection with parenteral schistosomiasis treatment in Egypt. BMC Infect Dis. 2002;2:29.
37. El Sherbini A, Mohsen SA, Hasan W, Mostafa S, El Gohary K, Moneib A, et al. The peak impact of an Egyptian outbreak of hepatitis C virus: has it passed or has not yet occurred? Liver Int. 2007;27(6):876–7.
38. Habib M, Mohamed MK, Abdel-Aziz F, Magder LS, Abdel-Hamid M, Gamil F, et al. Hepatitis C virus infection in a community in the Nile Delta: risk factors for seropositivity. Hepatology. 2001;33(1):248–53.
39. Medhat A, Shehata M, Magder LS, Mikhail N, Abdel-Baki L, Nafeh M, et al. Hepatitis c in a community in Upper Egypt: risk factors for infection. Am J Trop Med Hyg. 2002;66(5):633–8.
40. Arafa N, El Hoseiny M, Rekacewicz C, Bakr I, El-Kafrawy S, El Daly M, et al. Changing pattern of hepatitis C virus spread in rural areas of Egypt. J Hepatol. 2005;43(3):418–24.
41. Darwish MA, Faris R, Darwish N, Shouman A, Gadallah M, El-Sharkawy MS, et al. Hepatitis c and cirrhotic liver disease in the Nile delta of Egypt: a community-based study. Am J Trop Med Hyg. 2001;64(3-4):147–53.
42. Katsoulidou A, Sypsa V, Tassopoulos NC, Boletis J, Karafoulidou A, Ketikoglou I, et al. Molecular epidemiology of hepatitis C virus (HCV) in Greece: temporal trends in HCV genotype-specific incidence and molecular characterization of genotype 4 isolates. J Viral Hepat. 2006;13(1):19–27.
43. Henquell C, Guglielmini J, Verbeeck J, Mahul A, Thibault V, Lebray P, et al. Evolutionary history of hepatitis C virus genotype 5a in France, a multicenter ANRS study. Infect Genet Evol. 2011;11(2):496–503.
44. Stumpf MP, Pybus OG. Genetic diversity and models of viral evolution for the hepatitis C virus. FEMS Microbiol Lett. 2002;214(2):143–52.
45. Murphy DG, Sablon E, Chamberland J, Fournier E, Dandavino R, Tremblay CL. Hepatitis C virus genotype 7, a new genotype originating from central Africa. J Clin Microbiol. 2015;53 (3):967–72.

46. Salmona M, Caporossi A, Simmonds P, Thelu MA, Fusillier K, Mercier-Delarue S, et al. First next-generation sequencing full-genome characterization of a hepatitis C virus genotype 7 divergent subtype. Clin Microbiol Infect. 2016;22(11):947 e1-e8.
47. Kramvis A. Genotypes and genetic variability of hepatitis B virus. Intervirology. 2014;57 (3-4):141–50.
48. Norder H, Courouce AM, Coursaget P, Echevarria JM, Lee SD, Mushahwar IK, et al. Genetic diversity of hepatitis B virus strains derived worldwide: genotypes, subgenotypes, and HBsAg subtypes. Intervirology. 2004;47(6):289–309.
49. Pourkarim MR, Amini-Bavil-Olyaee S, Kurbanov F, Van Ranst M, Tacke F. Molecular identification of hepatitis B virus genotypes/subgenotypes: revised classification hurdles and updated resolutions. World J Gastroenterol. 2014;20(23):7152–68.
50. Kramvis A, Kew M, Francois G. Hepatitis B virus genotypes. Vaccine. 2005;23(19):2409–23.
51. Foley BT, Leitner T, Paraskevis D, Peeters M. Primate immunodeficiency virus classification and nomenclature: review. Infect Genet Evol. 2016;46:150–8.
52. Hauri AM, Armstrong GL, Hutin YJ. The global burden of disease attributable to contaminated injections given in health care settings. Int J STD AIDS. 2004;15(1):7–16.
53. Sharp PM, Bailes E, Chaudhuri RR, Rodenburg CM, Santiago MO, Hahn BH. The origins of acquired immune deficiency syndrome viruses: where and when? Philos Trans R Soc Lond Ser B Biol Sci. 2001;356(1410):867–76.
54. Gao F, Bailes E, Robertson DL, Chen Y, Rodenburg CM, Michael SF, et al. Origin of HIV-1 in the chimpanzee Pan troglodytes troglodytes. Nature. 1999;397(6718):436–41.
55. Tebit DM, Arts EJ. Tracking a century of global expansion and evolution of HIV to drive understanding and to combat disease. Lancet Infect Dis. 2011;11(1):45–56.
56. Takebe Y, Uenishi R, Li X. Global molecular epidemiology of HIV: understanding the genesis of AIDS pandemic. Adv Pharmacol. 2008;56:1–25.
57. Magiorkinis G, Angelis K, Mamais I, Katzourakis A, Hatzakis A, Albert J, et al. The global spread of HIV-1 subtype B epidemic. Infect Genet Evol. 2016;46:169–79.
58. McCutchan FE, Hoelscher M, Tovanabutra S, Piyasirisilp S, Sanders-Buell E, Ramos G, et al. In-depth analysis of a heterosexually acquired human immunodeficiency virus type 1 superinfection: evolution, temporal fluctuation, and intercompartment dynamics from the seronegative window period through 30 months postinfection. J Virol. 2005;79(18):11693–704.

Laboratory Diagnosis

4

Anna Maria Geretti, Mark Atkins, Eleni Nastouli, and Daniel Bradshaw

4.1 Historical Perspective

The field of viral hepatitis has a long history dating back to the 1960s with the initial descriptions of "serum" hepatitis and "infectious" hepatitis. For many years, the causative agent remained elusive. Tests to detect hepatitis A virus (HAV) and hepatitis B virus (HBV) were introduced in the 1970s. It became soon clear that the majority of cases of parenterally transmitted hepatitis were not in fact due to either HAV or HBV [1]. This resulted in the introduction of the term non-A, non-B hepatitis (NANBH), which for years remained a diagnosis of exclusion of other viral and nonviral causes. This challenging diagnostic scenario changed dramatically in the late 1980s, through the painstaking work of Michael Houghton, Harvey Alter, and Charles Rice, which led to the discovery of the hepatitis C virus (HCV) [2] and the development of the first solid-phase enzyme immunoassay (EIA) for the detection of antibodies against HCV [3]. In 2020, Houghton, Alter and Rice were jointly awarded the Nobel Prize in Physiology or Medicine for their discovery. This first-generation HCV antibody assay was designed in a simple, IgG anti-globulin format and was not without its limitations. To improve specificity, the recombinant

A. M. Geretti (✉)
Institute of Infection, University of Liverpool, Liverpool, UK
e-mail: geretti@liverpool.ac.uk

M. Atkins
Micropathology, Coventry, UK
e-mail: mark.atkins5@nhs.net

E. Nastouli
Department of Clinical Virology, UCL Hospital NHS Foundation Trust, London, UK
e-mail: eleni.nastouli@nhs.net

D. Bradshaw
Virus Reference Department, Public Health England, Colindale, UK
e-mail: Daniel.Bradshaw@phe.gov.uk

A. Hatzakis (ed.), *Hepatitis C: Epidemiology, Prevention and Elimination*,
https://doi.org/10.1007/978-3-030-64649-3_4

immunoblot assay (RIBA) was soon introduced as supplemental test to identify antibody reactivity to individual HCV antigens. The combination of first-generation EIA and RIBA revolutionised the diagnosis of HCV infection, clarified the epidemiology of NANBH, and for the first time enabled the screening of blood and blood products. Differentiating between active HCV infection and past exposure remained challenging until the advent of the polymerase chain reaction (PCR) that finally enabled detection of HCV RNA.

4.2 Markers of HCV Infection

HCV has a single-stranded positive-sense RNA genome of approximately 9.6 kilobases. A single open reading frame flanked by 5′ and 3′ untranslated regions (UTRs) encodes a polyprotein of 3037–3800 amino acids, which is cleaved into at least ten products, including structural proteins (core protein C; envelope glycoproteins E1 and E2), p7 (an ion channel), and nonstructural (NS) proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B). A reliable culture method is not available for HCV. The main markers used for diagnosis and treatment comprise those that directly detect and quantify the virus in a sample (HCV RNA, HCV core antigen), those that characterise the viral genome (genotype, drug resistance), and those that detect the immune response to infection (HCV antibody) (Table 4.1).

4.3 Diagnostic Algorithm

The classic approach for diagnosing HCV infection recommends screening of serum (or plasma) for HCV antibodies followed by direct virus detection to confirm active infection [4–6], typically a molecular assay to detect HCV RNA qualitatively or measure it quantitatively (Fig. 4.1). An alternative approach targets HCV core antigen (HCVcAg) (Fig. 4.2). It is recommended that virus detection is pursued as a reflex test using the same sample as the original antibody screening assay to streamline the diagnostic pathway and avoid delays whilst waiting for a second sample. In low prevalence settings, confirmation of HCV antibody reactivity (e.g. by a second assay) may be sought prior to applying direct virus detection methods. Alternatively, the strength of reactivity (signal-to-cutoff ratio) of the HCV antibody assay may be used to decide whether to progress immediately to direct virus

Table 4.1 Markers of HCV infection used in diagnosis and treatment

Marker	Diagnosis	Treatment
HCV total or IgG antibody	✓	
HCV RNA qualitative	✓	
HCV RNA quantitative	✓	✓
HCV core antigen	✓	(✓)
HCV genotype/subtype		✓
HCV drug resistance		✓

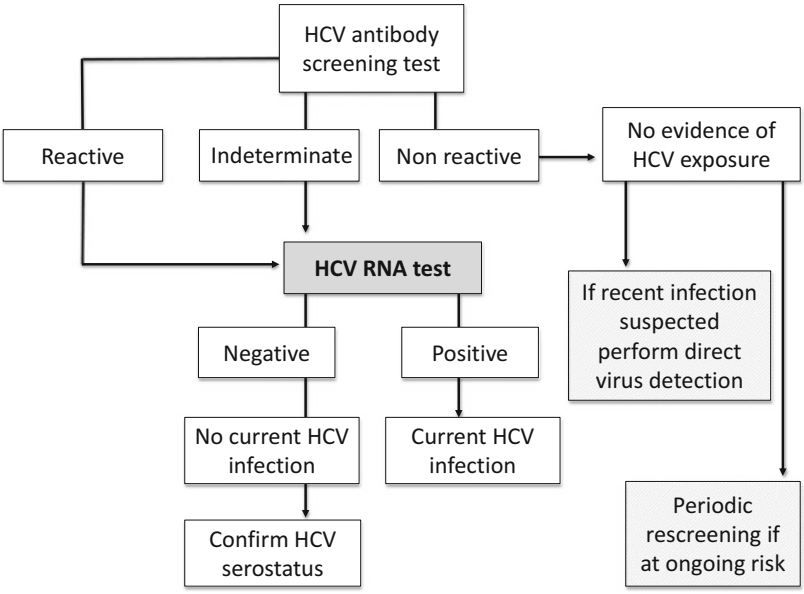


Fig. 4.1 Algorithm for HCV diagnosis using HCV RNA testing to demonstrate an active infection

detection, bearing in mind that cutoffs that predict HCV RNA positivity with >95% confidence are assay-specific (e.g. may range from 3.8 to 11.0 depending on the assay) and therefore require assay-specific validation.

4.3.1 Temporal Evolution of Diagnostic Markers

HCV RNA becomes detectable in peripheral blood within 1–3 weeks of infection, followed by HCVcAg within 2–8 days and by HCV antibodies within an average of 4–5 weeks (Fig. 4.3). HCV antibodies usually become detectable within about 9 weeks of infection, although occasionally may take 12 weeks or longer to develop. Seroconversion may be delayed in immunocompromised patients. In studies of HIV-positive individuals, two-thirds tested positive at 12 weeks, and 5% remained negative up to 1 year after infection [7].

4.3.2 Testing for HCV Antibodies

HCV antibodies are detected with high sensitivity and specificity using EIAs and chemiluminescence immune assays (CIAs). This has not always been the case. First-generation tests used antigens from NS4 alone and had poor specificity and sensitivity [8]. Sensitivity was only about 80% even in high prevalence populations, and false-positive rates were as high as 70% in low prevalence populations such as blood

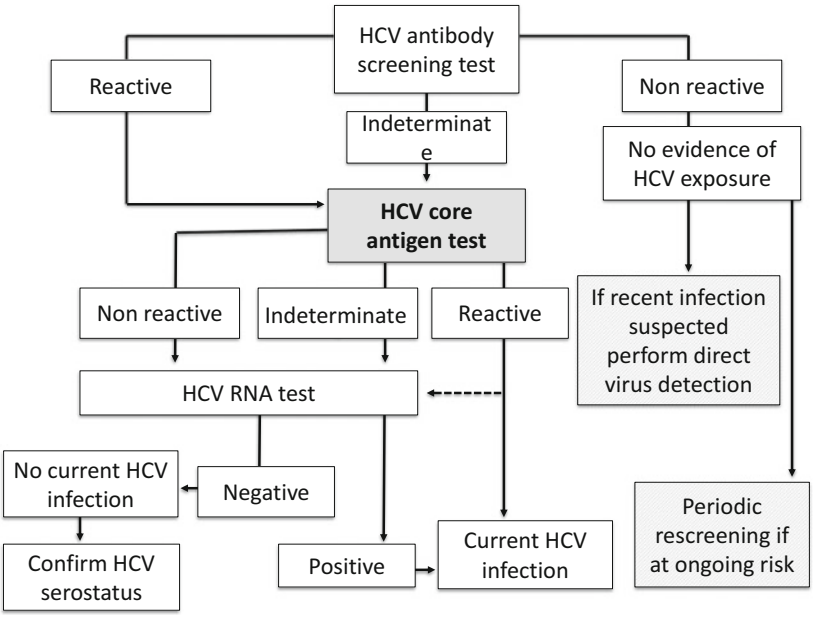


Fig. 4.2 Algorithm for HCV diagnosis using HCV core antigen (HCVcAg) testing to demonstrate an active infection. Although a positive HCVcAg test may be considered conclusive evidence of a current HCV infection, adding a quantitative HCV RNA test, where feasible, increases confidence in the result whilst also providing the pre-treatment viral load

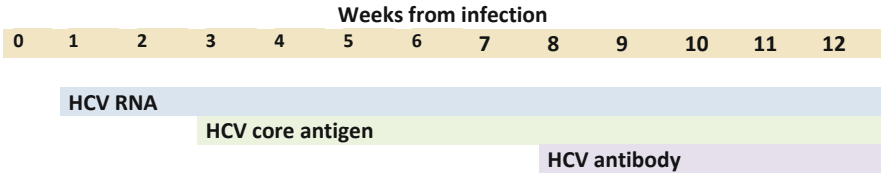


Fig. 4.3 Temporal evolution of diagnostic HCV markers, including HCV RNA, HCV core antigen, and HCV antibodies

donors. Seroconversion in patients with acute HCV infection was often not detected until 3 months or longer after infection. Improvements to assay performance were achieved by the addition of further HCV antigens. Second-generation assays, introduced in 1991, incorporated antigens from core, NS3, and NS4, which increased sensitivity (up to 95%) and specificity and decreased the diagnostic window period for detecting seroconversion [8]. The latest, third-generation assays utilise multiple recombinant or synthetic viral antigens from core, NS3, NS4, and NS5. Sensitivity is between 98% and 100% in immunocompetent populations with and without liver disease, and the diagnostic window period for detecting seroconversion has been reduced to 45–70 days [9]. The assays are well adapted to high-throughput, automated, random access analysers that are widely available in

diagnostic laboratories, and are relatively inexpensive and easy to perform, with a test time of 30 min or less. Assay sensitivity is reduced (50–95%) in immunocompromised patients and those on haemodialysis [10]. In circumstances where false antibody negativity is suspected, screening must employ direct virus detection methods.

4.3.2.1 Confirmation of HCV Serostatus

Subjects that test reactive for HCV antibody by the screening EIA or CIA but lack detectable HCV RNA or HCVcAg may have either a past HCV infection or a false-positive screening test. Confirmation of serostatus is desirable to enable appropriate counselling. Even at 99% assay specificity, false-positive results progressively outnumber true-positive results as HCV prevalence in the screened population declines below 1% [11]. Immunoblot assays provide a supplementary method for specific antibody detection. RIBA was discontinued in 2011. The INNO-LIA® HCV Score by Fujirebio (previously Innogenetics) is a third-generation immunoblot assay which incorporates recombinant proteins and synthetic peptides derived from core, E2, NS3, NS4A, NS4B, and NS5A fixed as six discrete lines on a nylon membrane along four control lines. Results are reported as negative (all HCV antigen lines are negative), positive ($\geq$2 HCV antigen lines show $\pm$ or higher reactivity), or indeterminate (only one HCV antigen line shows $\pm$ or higher reactivity for NS3 or 1+ or higher reactivity for other HCV antigens). Simpler algorithms to confirm HCV serostatus have been explored which omit the lengthy and expensive immunoblot assays. Using a second HCV antibody EIA or CIA can significantly reduce the total number of false-positive tests [11]. Despite these shortcomings, HCV antibody detection using serological methods remains the mainstay of the laboratory-based diagnosis of HCV infection for the majority of patients.

4.3.2.2 HCV Antibody Testing in Sub-Saharan Africa

EIAs and CIAs that run in parallel on the same sample can give discrepant results in populations in sub-Saharan Africa. In a study investigating HCV RNA-negative adults in Ghana, prevalence of HCV antibody by three widely available commercial assays ranged between 7.5% and 28.4% when applying the manufacturer' recommended interpretative cutoffs for each assay, declining to 3% when considering samples reactive in all three assays, and to 1.5% after supplementary antibody testing by INNO-LIA [12]. Similar data have been reported from other regions in Africa [13, 14], casting doubt over the reliability of HCV prevalence estimates for sub-Saharan Africa that are based upon a single antibody screening test. It has been proposed that persistent infections that trigger production of autoantibodies (e.g. with *Schistosoma*) may cause cross-reactivity in these populations. Applying higher interpretative cutoffs can reduce the number of samples requiring expensive and time-consuming confirmatory testing in these settings [12].

4.3.3 Testing in Recent Infection

As maturation of antibody responses to HCV occurs slowly after transmission (Fig. 4.3), direct screening for HCV RNA or HCVcAg reduces the diagnostic window and is indicated when a recent HCV infection is suspected. Immune assays that simultaneously target HCV antibodies and core antigen have become available commercially, although relative sensitivity and specificity remain to be firmly established. The combination assays are less sensitive than assays that target only HCVcAg; however, they may be effective in improving the safety of blood donations in resource-limited settings where HCV RNA detection is neither affordable nor technically possible [15]. Most patients newly diagnosed with HCV are positive for both HCV antibody and HCV RNA at the time of the initial presentation. Unless a previous result is available, it is not possible to determine whether the infection was acquired recently using routine diagnostic methods. HCV IgM responses show variable patterns of detection in HCV infection and can be found in patients with acute HCV infection but also in many patients with chronic HCV infection [16]. Serial IgM measurements over time and testing for HCV IgG avidity can help determine whether the infection is recent [17]. However, such tests have yet to find much clinical utility and are not part of routine diagnostic practice.

4.3.4 Diagnosing Reinfection

HCV antibodies remain detectable long-term in patients who clear the infection either spontaneously or as a result of antiviral therapy, and direct virus detection methods are required to diagnose a reinfection. HCV reinfection is defined by the reappearance of HCV RNA after evidence of clearance and requires the demonstration that infection is due to a different HCV strain rather than being reflective of a post-treatment relapse. If the genotype is the same, viral genome sequencing and phylogenetic analysis can be used to compare the previous and current HCV strain. This requires the availability of either a stored sample (serum or plasma) or a viral sequence (obtained for genotyping or drug resistance testing) from the previous infection. However, this approach cannot distinguish between a post-treatment relapse and reinfection from the same source.

4.3.5 Testing for HCV RNA

The presence of HCV RNA in peripheral blood is a reliable marker of HCV replication. To confirm active infection, HCV RNA can be detected either qualitatively or quantitatively. Several molecular assays are commercially available that typically employ real-time PCR or TMA (transcription-mediated amplification) on fully or partially automated platforms. The assays require specialised laboratory staff and infrastructure, including expensive equipment and reagents, and dedicated procedure areas. Blood samples must be processed as soon as possible after

Table 4.2 Terminology of HCV RNA testing

Term	Definition
Viral load	HCV RNA level in serum or plasma reported in IU/mL
Lower limit of quantification (LLQ)	The lower limit of the assay validated linear quantification range; below this level, HCV RNA detection is possible but accurate quantification is not possible
Lower limit of detection (LLD)	The HCV RNA level that results in a detection signal at a rate of ≥95%; below this level, the rate of signal detection declines progressively as HCV RNA levels approach zero
HCV RNA/target detected	HCV RNA detected below the LLQ
HCV RNA/target not detected	No detection of HCV RNA; if any HCV RNA is present, it is at levels below the LLD

collection to separate serum or plasma, which is then either tested immediately or stored at −20 °C or −70 °C to minimise RNA loss [18]. The assays are typically standardised using a common reference panel, and results are reported in international units (IU) to facilitate cross-assay comparison. Over 98% of treatment-naïve patients with chronic HCV infection have HCV RNA levels >4 $\log_{10}$ IU/mL [19]. As a result, the World Health Organization (WHO) considers qualitative or quantitative HCV RNA assays with a lower limit of detection of 3000 IU/mL acceptable for screening purposes [20]. Standard laboratory practice however is to use assays that provide both a sensitive confirmation of active infection and an accurate quantification of the viral load (Table 4.2). In clinical trials, the preferred test has historically been either the Roche Cobas TaqMan HCV version 2.0 assay or the Abbott RealTime HCV assay which employ real-time PCR; new versions include the Roche cobas® HCV Test with a lower limit of quantification (LLQ) of 15 IU/mL. The Hologic Aptima HCV Quant Dx assay employs TMA on the Panther platform with an LLQ of 10 IU/mL. These assays generally yield highly comparable results across major HCV genotypes [21]. However, discrepancies have also been reported, with some measurements between tests varying by more than 1 $\log_{10}$. Vigilance of test performance is important considering the high genetic variability of circulating HCV strains, as nucleotide mismatches can lead to substantial viral load underestimations with some variants [22].

4.3.6 Testing for HCV Core Antigen

HCVcAg is a multifunctional protein composed of three domains that forms the viral nucleocapsid and by interacting with lipid droplets, the viral RNA, and the endoplasmic reticulum is thought to play an important role in disease pathogenesis [23]. Immune assays for the detection of HCVcAg were developed in the late 1990s, following production of the first anti-core monoclonal antibody [24]. Although the assays initially had limited sensitivity, performance improved in subsequent generations. HCVcAg testing currently provides a simple and rapid

method for confirming active HCV infection, which is usually less expensive than testing for HCV RNA. The assay can be run as a reflex test on the same high-throughput platforms used for HCV antibody screening. Compared with HCV RNA detection, HCVcAg testing is at least 60% sensitive and 83% specific in diagnosing HCV infection, with possible differences in sensitivity by HCV genotype [24–28]. It has been proposed that 1 pg/mL of HCVcAg equals to approximately 8000 IU/mL of HCV RNA for genotype 1. The HCVcAg to HCV RNA ratio, however, differs between patients and within the same individual at different time points during the infection, suggesting that the core protein can exist in peripheral blood without necessarily being associated with viral RNA. With the Abbott Architect test, an automated CIA for the detection and quantification of HCVcAg, a good nonlinear correlation has been observed with HCV RNA levels measured by the Abbott RealTime HCV load assay. The lower limit of detection corresponds to an HCV RNA load of 500–3000 IU/mL depending on the genotype. Due to lower sensitivity, to achieve 100% detection of active infection, HCV antibody-positive/HCVcAg-negative samples require confirmation of HCV RNA negativity. Such a biphasic strategy of reserving HCV RNA testing only for patients who test HCVcAg negative can be cost-effective for diagnosing HCV infection and is potentially feasible for facilitating HCV diagnosis in settings with limited infrastructure for molecular testing [29, 30].

4.3.7 Evolving Screening Strategies

An estimated 1% of the world population is chronically infected with HCV, and populations who have poor healthcare outcomes, have low healthcare system engagement (through access or behaviours), and are socioeconomically deprived are disproportionately affected [31]. With the growing availability of curative antiviral therapy, WHO has set the global target of eliminating HCV infection as a major public health threat by 2030 [31]. Whilst periodic HCV screening is recommended in all subjects at high risk of infection, less than half of all HCV carriers are aware of their status, and a substantial expansion of testing is required to meet the WHO target of 90% diagnosed by 2030. In the United States, one-time screening has been recommended by the Centers for Disease Control and Prevention for all adults born between 1945 and 1965 [32], an approach that has been found to be effective in identifying previously undiagnosed HCV infections [33]. One-time screening of persons 18 and older has been proposed to identify more persons with HCV than the birth cohort approach, and to be cost-effective, leading to improved clinical outcomes [34]. Opt-out screening has been applied in a variety of settings where people with undiagnosed HCV infection may present, including prisons, emergency departments, and primary care and community settings [35, 36]. Innovative sampling and testing approaches are required to support such efforts. Use of dried blood spots (DBS) produced from capillary blood collected by finger-prick has been shown to facilitate sample collection and transport to the laboratory. Tests have also become available for use at the point of care, including fully contained,

single-step molecular assays that detect HCV RNA in less than 1-2 hours. These technological advances offer the opportunity to simplify access to rapid and accurate HCV testing, improving the number of people diagnosed and treated [37].

4.3.7.1 Alternative Sampling and Testing Methods

Traditional HCV testing involves collection of blood by venepuncture. Collecting capillary blood by finger-prick allows sampling outside of healthcare facilities and in people with poor venous access. The blood can be spotted on filter paper to produce DBS [38, 39]. DBS are relatively stable over time, can be stored and transported at room temperature, and can be shipped via regular mail or courier services (Table 4.3). In the laboratory, the filter paper is immersed in a buffer to allow elution of the blood specimen over several hours or overnight, and the eluate is then used as a standard sample for HCV testing, including measurement of HCV antibody, HCVcAg, and HCV RNA. The eluate can also be used for sequencing HCV RNA [40]. The efficiency of DBS for HCV antibody detection is only slightly lower than that of serum specimens, with a pooled sensitivity and specificity of around 98% and 99%, respectively. Pooled sensitivity and specificity are both around 98% for qualitative HCV RNA detection, whereas sensitivity drops to around 76% for HCVcAg [41]. Pooling DBS (up to two 6 mm punches from up to five DBS) during the elution step can reduce cost and labour when screening large number of samples for the presence of HCV RNA, without significantly affecting qualitative HCV RNA detection in treatment-naïve populations [40]. Thus, available evidence indicates that DBS offer a reliable diagnostic tool that can facilitate access to HCV testing, including qualitative HCV RNA detection to confirm an active infection. Assay manufacturers need to validate the use of their assays with DBS specimens and provide technical guidance regarding their use. There remain important research needs to improve standardisation and implementation and to evaluate use of DBS in monitoring antiviral treatment (Table 4.4).

4.3.7.2 Point-of-Care Testing

Patients and healthcare professionals tend to favour testing with immediate test results over phlebotomy with test results given at follow-up [42, 43]. HCV antibody

Table 4.3 Dried blood spots (DBS) for HCV diagnosis

Advantages	Disadvantages
• No venepuncture required	• Require transport to the laboratory for testing
• Allow sampling outside of healthcare facilities	• Each assay must be calibrated and validated for use with DBS
• Facilitate sample transport to the laboratory	• Add processing cost
• Facilitate sample storage	• Less sensitive than testing serum or plasma
• Allow testing for multiple HCV markers	• Require follow-up for returning results to patients
• Allow detection of other analytes (e.g. HIV, HBV)	• Pose a low but still present biohazard

Table 4.4 Research needs in the use of dry blood spots for HCV diagnosis and monitoring

• Establish diagnostic accuracy under a range of real-life sampling, storage, and transport conditions
• Establish clinically relevant performance cutoffs for diagnosis
• Increase programmatic experience across a wide range of testing settings
• Assess impact on uptake of testing, case identification, and linkage to treatment
• Evaluate the impact of co-infections and immunosuppression on performance
• Determine and validate use for monitoring treatment responses including threshold for detection and how it is affected by sampling, transport, storage, and testing conditions

can be detected at point of care using either oral mucosal transudate (OMT) or capillary blood collected by finger-prick, taking 20–40 min to produce a result. There is some evidence that people may find rapid salivary testing more attractive than finger-prick testing. The OraQuick rapid HCV test offers a sensitivity and specificity of 89.9% and 100% when using OMT [44]. Sensitivity improves to 98.8% with finger-prick blood. Importantly, the sensitivity in OMT is higher (97.2%) in HCV-seropositive patients who are viraemic as compared to non-viraemic individuals (82.2%); extension of the read time to 40 min may enhance sensitivity with OMT in non-viraemic patients. In contrast, there appears to be no significant differences in sensitivity between viraemic and non-viraemic individuals when testing finger-prick blood. One downside is that the OraQuick test is about ten times the price of laboratory-based serology tests, which represents a significant barrier to increased use.

Point-of-care tests that rely on antibody detection require follow-up testing for confirmation of carrier status by detection of HCV RNA or HCVcAg, which makes the diagnostic algorithm cumbersome. Until recently, molecular testing for viral nucleic acid was confined to specialised laboratory facilities, requiring highly trained personnel and a rigorous application of measures to prevent contamination during the separate steps of sample preparation and nucleic acid extraction, amplification, and detection. Newer cartridge-based systems combine these separate steps into a single, self-contained process that is easy to perform by nonspecialist personnel and has a low risk of cross-contamination between specimens, thus offering a major diagnostic advance that is suitable for adoption in both the laboratory and outside of the laboratory, and both within and outside of healthcare settings (Table 4.5). The Cepheid GeneXpert system is a modular platform that performs nucleic acid amplification and detection of each sample within a self-contained individual cartridge [45–47]. The standard HCV RNA testing protocol requires 1 mL of sample. Applicability for HCV RNA screening in small volume (100 μL) capillary blood collected by finger-prick was investigated among 210 subjects recruited from a variety of community settings in Australia [46]. The study reported excellent sensitivity and specificity relative to the Abbott RealTime HCV assay. Further validation data for use with finger-prick blood demonstrate good assay performance across the major HCV genotypes (Fig. 4.4), a high degree of concordance with results obtained in paired venous blood, stability of HCV RNA detection over time, and a low failure

Table 4.5 Comparison of methods for direct HCV detection

Target	HCV RNA	HCV RNA	HCV RNA	HCVcAg	HCVcAg
Sample	Venous blood	Dry blood spot	Capillary or venous blood	Venous blood	Dry blood spot
Stringency of sampling	High	Moderate	Moderate	Moderate	Moderate
Method	PCR or TMA	PCR or TMA	PCR or TMA	Immune assay	Immune assay
Testing setting	Laboratory	Laboratory	Point of care	Laboratory	Laboratory
Laboratory infrastructure	Highly developed	Highly developed	Simple	Developed	Developed
Turn around	Hours to days	≥12 h with elution time	<2 h	<1 h	≥12 h with elution time
Batching sample required	Reduces cost on some platforms	Reduces cost on some platforms	No	No	No
Standardisation	Highest	Moderate	Higher for venous blood	High	Lowest
Sensitivity	+++++	++	++++	+++	+

rate when the assay is run by nonspecialist healthcare personnel. Overall, the assay failure rate is in the region of 1% and can be reduced by ensuring sufficient volume of blood and diluent are added to the cartridge. Recent progress in cartridge technology have further shortened testing times on GeneXpert ensuring that individuals can receive results within 1 h of sampling, making adoption in nonhospital locations more attractive [47]. One limitation is that the Cepheid Xpert cartridge contains guanidinium thiocyanate as the lysis agent, which is highly toxic and therefore requires special handling and disposing. It should be noted also that using small-volume samples reduces assay sensitivity relative to testing a larger sample volume, which may make the finger-prick technique less suitable for demonstrating a sustained virological response (SVR) after completion of antiviral therapy. Further evaluation of this attractive application is required. The Genedrive platform is a handheld 600 g portable thermocycler for nucleic acid detection that can be battery operated and requires only 30 μL of sample. The qualitative HCV RNA assay identifies all major HCV genotypes with 98.6% sensitivity and 100% specificity, whilst the lower limit of detection is 2362 IU/mL relative to the Abbott RealTime HCV assay. These properties make the assay suitable for decentralised and field-based HCV testing [48], provided however serum or plasma can be obtained. Other point-of-care molecular platforms are also in advanced development, such as Alere q (Alere), EOSCAPE (Wave 80 Biosciences), PanNAT platform (Micronics), Truelab PCR (Molbio Diagnostics), and RT CPA (Ustar

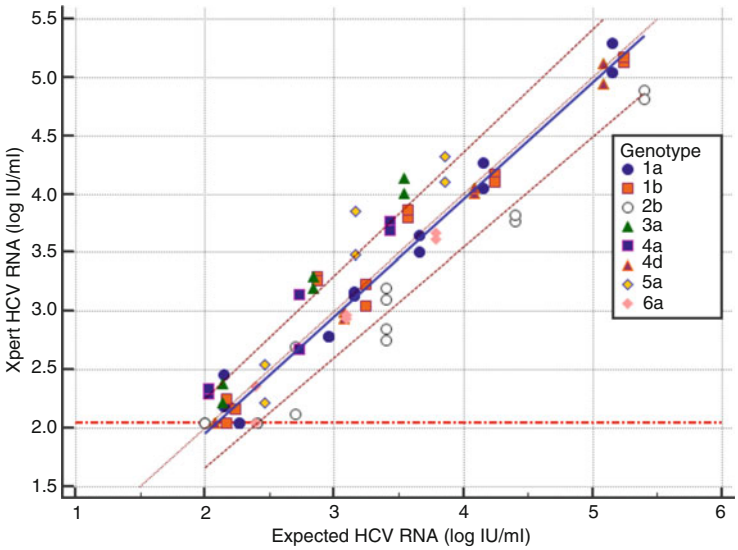


Fig. 4.4 Scatterplot of Xpert HCV RNA load results ($\log_{10}$ IU/mL) with a validation panel representing HCV genotypes 1a, 1b, 2b, 3a, 4a, 4d, 5a, and 6a. Serial dilutions were tested in duplicate according to the small-volume protocol, whereby 100 μL was taken from each dilution and made up to 1.1 mL in HCV RNA Xpert diluent prior to testing. A total of 82 samples were tested. The lines of equality (diagonal dotted) and Passing-Bablok regression (diagonal solid) bordered by 95% confidence intervals (diagonal dashed) are shown. The theoretical small-volume protocol lower limit of quantitation is represented by the horizontal dashed line. There were 24 samples representing the dilution point of 2 $\log_{10}$ IU/mL (actual HCV RNA levels were 140, 149, 100, 120, 108, 110, 290, and 247 IU/mL for genotypes 1a, 1b, 2b, 3a, 4a, 4d, 5a, and 6a, respectively). Of these 24 samples, 12 showed quantifiable HCV RNA levels (>110 IU/mL; median 189 IU/mL; range 145–355 IU/mL); the other 12 samples showed qualitative HCV RNA detection (estimated HCV RNA levels >44 and <110 IU/mL). Median standard deviations (IQR) of duplicate HCV RNA measurements in these experiments were 0.10 (0.02–0.17), 0.04 (0.03–0.05), 0.07 (0.06–0.07), 0.07 (0.03–0.09), 0.19 (0.12–0.26), 0.05 (0.03–0.06), 0.20 (0.18–0.23), and 0.03 (0.03–0.04) for genotypes 1a, 1b, 2b, 3a 4a, 4d, 5a, and 6a, respectively. $R^2 = 0.901$, $y = 0.3339 + 0.9035\,x$

Biotechnologies). These technical improvements will enhance feasibility of one-stop diagnosis of HCV infection at point of care, ensuring that results and appropriate care can be delivered promptly. Careful implementation is required to ensure high uptake and preserve quality, adopting assurance processes like those applied to laboratory settings.

4.4 Guiding and Monitoring Treatment

Appropriate selection and monitoring of antiviral therapy for HCV may consider several virological factors, including the HCV RNA load, HCV genotype and subtype, and presence of drug resistance (Table 4.6; Fig. 4.5).

Table 4.6 Recommended assessments to guide and monitor antiviral therapy

Target	Use in relation to antiviral therapy	Indication
Viral load	• At any time prior to starting • At week 2–4 after starting • At week 6 after starting • At completion • At week 12 after completion • At week 24 (or later) after completion	• All • Optional • Optional • Optional • All • Desirable
Genotype/subtype	• At any time prior to starting	• All
Drug resistance	• Prior to starting • At failure, prior to retreatment	• Selected cases • Desirable

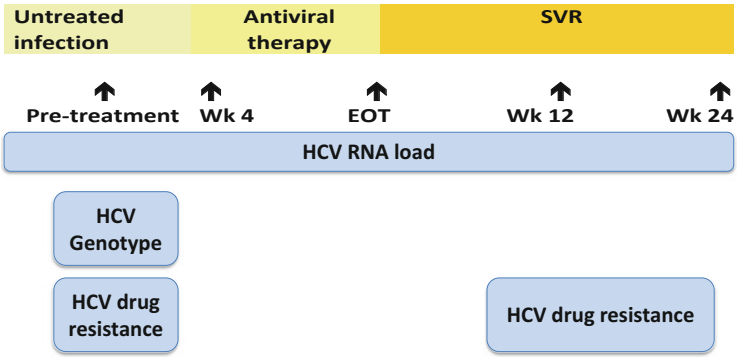


Fig. 4.5 Virological tests to guide and monitor antiviral therapy. *EOT* end of treatment, *SVR* sustained virological response

4.4.1 Viral Load

Spontaneous virus clearance is rare beyond 4 months of infection, and persistence of HCV RNA after 6 months indicates a chronic infection. In the chronic phase, HCV RNA levels remain relatively stable in most untreated patients, whilst approximately 15% experience fluctuations greater than 1 $\log_{10}$ IU/mL (>10-fold) [49]. There is no direct correlation between viral load and severity of liver disease or risk of liver disease progression. However, pre-treatment viral load levels can influence responses to antiviral therapy, as shown, for example, with elbasvir/grazoprevir or with sofosbuvir plus daclatasvir in people with HIV [50, 51]. Patients require HCV RNA quantification prior to starting treatment, but there is no need for repeat viral load measurements outside of those required to guide and monitor antiviral therapy. On treatment, viral load measurements are often taken at week 2–4 as an indicator of early response and adherence [52–55]. In clinical trials of direct-acting antiviral agents (DAAs), almost all patients without cirrhosis achieved an undetectable HCV RNA level at week 4, whereas patients with cirrhosis may need longer than 4 weeks. However, there are limited data to inform interpretation of week 2–4 viral load, and clinical judgement is required to interpret viral load patterns. Whilst achieving an

undetectable HCV RNA early after treatment initiation is a positive predictor of treatment success, showing a detectable viral load at this point does not necessarily indicate failure or that treatment should be discontinued. Where there is evidence of a suboptimal response, expert opinion indicates that the viral load should be repeated at week 6 and treatment should be discontinued if an increase by >1 $\log_{10}$ IU/mL is observed relative to the earlier on-treatment measurement [52]. The significance of an HCV RNA test result at week 4 that remains positive but at a lower level at week 6 is unknown, and no guidance is available to inform treatment decisions. HCV RNA levels at the end of treatment (EOT) are helpful to differentiate relapse from non-response/breakthrough in patients who fail to achieve an SVR. However, HCV RNA detection at very low levels at EOT is not necessarily associated with treatment failure and is not used as an indication that treatment should be extended [52]. Assessing virus clearance from peripheral blood is required to establish a cure, currently defined as undetectable HCV RNA at week 12 after completion of therapy using an assay of high sensitivity (15 IU/mL or lower) [52, 53]. Overall, week 12 and week 24 results are >99% concordant and relapse beyond 24 weeks occurs in <0.2%. Although virological relapse is rare past week 12, a repeat test should be considered at week 24 (or later) after completing treatment, especially in patients with cirrhosis, or those who show an increase in hepatic transaminase levels.

4.4.2 Simplified Approaches

The desire to simplify the monitoring algorithm and facilitate expanded access to antiviral therapy informs the guidance that the pre-treatment, week 2–4, and EOT viral load measurements can be omitted and that inability to measure the viral load at these time points should not be a reason to delay or discontinue therapy [52, 53]. HCVcAg has also been proposed as an alternative to HCV RNA testing for determining treatment responses when HCV RNA testing is not available or not affordable [25, 56–59]. HCVcAg reactivity may take longer to clear than HCV RNA during antiviral therapy, probably because circulating core protein is available not only from virions but also from antigen-antibody complexes that have a longer half-life. Whilst further studies are required to establish reliable algorithms, testing for HCVcAg first, followed by HCV RNA testing of HCVcAg-negative samples, can eliminate as many as 75% of HCV RNA tests when investigating relapse after completion of antiviral therapy. Further studies are also needed to determine the applicability of HCV RNA detection in DBS and at point of care in this context.

4.4.3 HCV Genotype

4.4.3.1 Viral Genetic Diversity

HCV strains are classified into eight major genotypes that differ by 31–34% in their nucleotide sequence. Each genotype is further subdivided into subtypes, which may differ by 13–20% in their nucleotide composition [60]. Although HCV genotype

1 predominates globally, genotypes 2–6 are also highly prevalent and may be the main circulating type in different geographical regions or patient populations. Within genotypes and subtypes, further genetic diversification is observed at the population level, leading to the emergence and spread of distinct variants, including some with intrinsic resistance to one or more antiviral agent (e.g. the NS3 variant Q80K). Highly diverse, less characterised strains circulate in sub-Saharan Africa and in some regions of Asia, including variants of genotypes 1, 3, 4 and 6 with intrinsic resistance to some DAAs. HCV recombinants have also been described that represent chimeras of multiple genotypes and subtypes. In addition to true chimeras, mixed infections are observed at a variable but overall low prevalence (<8%) [61]. In one study, 6.7% of individuals diagnosed with genotype 3 also harboured genotype 1a strains [61]. Rates may be higher in certain populations such as injecting drug users [62]. Some of the studies using PCR-based next-generation sequencing (NGS) have reported high prevalence of mixed infections; however, interpretation of the findings is uncertain due to the technical limitations of NGS, especially when viral species are infrequent in a sample [61].

4.4.3.2 The Need for Genotyping

First- and second-wave direct-acting antiviral agents are genotype-dependent and therefore reliant on accurate HCV typing methods. Introduction of pan-genotypic antiviral agents will potentially minimise the need for sophisticated virological testing [63], which is usually unavailable in poorer regions or outside of specialist services. Currently, knowledge of genotype, and in some cases subtype, remains an integral part of selecting the most appropriate treatment regimen and duration of treatment, and whether this will change in the future remains to be seen. It is also important to note that the significance of viral genetic diversity in influencing treatment outcomes has been most extensively studied with dominant HCV genotypes and subtypes within clinical trials. As treatment availability expands globally, it will be important to monitor responses in patents carrying virus strains that have been less well characterised.

4.4.3.3 Genotyping Methods

HCV genotype and subtype testing is available commercially using one of the three main methodologies: reverse hybridisation (Versant LiPA HCV Genotype 2.0 Assay by Siemens) [64], Sanger sequencing (Trugene HCV 5'NC genotyping kit by Siemens) [65], and real-time PCR (RealTime HCV Genotype II Assay by Abbott; cobas® HCV GT by Roche [66, 67]). Trugene, a sequencing assay that targets the 5'UTR region of the viral genome, is labour-intensive and expensive and requires specialised infrastructure and personnel. Strip-based reverse hybridisation methods are relatively inexpensive, but remain labour-intensive, whereas real-time PCR assays are automated, more rapid, and less technically demanding. Commercial tests tend to be reproducible and have high degrees of concordance for the assignment of the major genotypes; however, performance is less satisfactory when considering detection of rare genotypes, discrimination between subtypes, and

recognition of mixed and recombinant infections. In these cases, additional tests are often required to avoid critical errors in genotype/subtype assignment [68–71].

Viral genome sequencing and phylogenetic analysis is the gold standard for determining the HCV genotype and subtype. Population (Sanger) sequencing is the current reference standard [71], and is generally widely available and sufficiently standardised in well-resourced laboratories, although it requires specialist personnel and is labour-intensive and expensive. Many laboratories resort to in-house protocols. Selection of the appropriate genome regions is important; this should usually include NS5B, whereas targeting 5'UTR alone should be avoided. When comparing the Trugene assay with NS5B sequencing as a reference, discrepant results can occur with 16% of samples. The Versant assay, in addition to the 5'UTR region, also targets the core region, reducing genotype/subtype misclassifications to about 6–11% relative to NS5B or core sequencing. One specific concern emerged with the discovery of HCV genotype 2/1 chimeras, which were identified as genotype 2 by commercial platforms, but showed the antiviral treatment response profile of genotype 1. One study investigated 442 blood samples from Italy, Germany, and Israel that were initially identified as HCV genotype 2 by commercial platforms and found that 61 were in fact 2k/1b, 2a/1b, or 2b/1a chimeras by sequencing. Treatment with sofosbuvir plus ribavirin, as indicated for genotype 2, resulted in failure in 25/27 (93%) patients, whereas most patients responded to regimens appropriate for genotype 1 [72].

NGS is a high-resolution alternative to Sanger sequencing, but requires appropriate standardisation, bioinformatics and interpretation cutoffs. The Sentosa SQ HCV Genotyping Assay (VELA Diagnostics) is a novel NGS-based test targeting NS5B and based on the Ion Torrent technology for deep sequencing which is undergoing technical development [73]. Deep sequencing-based assays are likely to become the method of choice for HCV subtyping in the future and may facilitate full-length genome sequencing to provide greater resolution relative to targeting specific regions of the viral genome.

4.4.4 HCV Drug Resistance

Testing for HCV drug resistance is accomplished by sequencing the viral genome to enable detection of resistance-associated variants that carry mutations known to confer reduced phenotypic susceptibility in vitro and/or to affect treatment responses in vivo [74]. Phenotypic resistance testing is only available in research settings and does not have a role in routine diagnostic practice.

4.4.4.1 Mechanisms and Principles of Drug Resistance

Following transmission of one or a few virus variants from a source, founder strains undergo genetic diversification in the new host as a result of the low fidelity of the viral RNA-dependent RNA polymerase (RdRp), the enzyme responsible for replicating the viral genome. The enzyme's high error rate (2.5×10^{-5} mutations per nucleotide per genome replication) [75], in conjunction with a high turnover and

Table 4.7 Terminology of HCV drug resistance testing

Term	Definition
Quasispecies	Diverse virus progeny that emerges within each host through virus genetic diversification
Barrier to resistance	Composite of multiple factors that modulate the emergence of resistance to a drug or a regimen
Resistance-associated substitutions (RAS)	Mutation in the viral genome that reduces drug susceptibility, expressed as letter (drug-susceptible amino acid), a number (amino acid position in the protein sequence), and a letter (substituted amino acid conferring drug resistance); e.g. NS5A Y93H indicates that at position 93 of the NS5A protein, tyrosine has been replaced by histidine
Drug-specific RAS	Mutation conferring reduced susceptibility to one particular antiviral agent
Class-specific RAS	Mutation conferring reduced susceptibility to $\geq$2 agents in the same class although not necessarily reducing susceptibility to all drugs of that class
Resistance-associated variants	Viral strains carrying one or more RAS
Selective pressure	Effect of virus replication during antiviral drug therapy driving emergence and genetic evolution of resistant variants
Fitness	Ability of a virus strain to replicate and infect
Compensatory mutations	Mutations in the viral genome that partially or fully restore the fitness of resistant variants
Fold-change	Increase in drug level required to inhibit virus replication in vitro relative to a drug-sensitive control strain, typically expressed as fold-change in IC50 (50% inhibitory concentration)

progeny number (10^{12} virions/day), eventually result in a large number of viral variants coexisting within the same host, termed quasispecies (Table 4.7). Mutations also occur spontaneously in the targets of antiviral therapy and can confer drug resistance [76]. Some of the spontaneously emerging mutations significantly impair viral fitness, and the mutated variants may struggle to replicate. Others have a negligible impact on viral fitness and may become established as dominant strains within the quasispecies and as lineages within populations [77]. In treatment-naïve patients, resistance-associated substitutions (RAS) affecting NS3, NS5A, and non-nucleoside NS5B inhibitors are common, albeit detected at varying prevalence rates depending on viral genetic region and genotype and the population studied. For example, in NS5A, the RAS A30K and Y93H occur in 9% and 12% of genotype 3 samples from the United Kingdom, and a subset of samples has paired RAS (A30K + L31M; A30K + Y93H) and show high levels of resistance to daclatasvir, velpatasvir, elbasvir, and pibrentasvir [78]. By contrast, RAS affecting nucleotide analogue inhibitors of the NS5B polymerase enzyme are rare (1–3%), as substitutions in the highly conserved active site of enzyme may effectively halt viral replication [79, 80]. RAS that are present at low frequency in the quasispecies become enriched by positive selection if virus replication occurs under drug pressure and, with ongoing selective pressure, become detectable by routine techniques

[81]. HCV RAS are nearly universal at the time of treatment failure. With ongoing selective pressure, the resistant variants continue to evolve acquiring additional mutations that increase resistance and cross-resistance. Mutations also emerge that partially or fully compensate for any reduced fitness conferred by RAS, thus restoring the virus ability to replicate. Once treatment is discontinued and selective pressure ceases, the resistant varinats may lose their advantage and be gradually outgrown by fitter variants lacking the mutations. The speed of reversion is inversely related to the fitness cost of the mutations. By available testing methodologies, RAS affecting the NS3 inhibitors become undetectable within a few months of stopping NS3 inhibitors. The significance of such disappearance has been questioned however [82]. Some resistance pathways, especially those involving NS5A inhibitors, result in resistant variants that are highly fit and persist long-term in the absence of drug-selective pressure [83]. At the other extreme, variants with reduced susceptibility to nucleoside analogue NS5B inhibitors bear a large fitness cost that is not easily compensated by additional mutations. Thus, these NS5B mutants emerge infrequently on therapy, and disappear rapidly off therapy, limiting their impact on treatment outcomes [84].

4.4.4.2 Which Test to Use

Genotypic susceptibility testing is the recommended test as it is widely available in well-resourced laboratories, although there remains a need for greater standardisation [85]. Either a fragment or the full length of the HCV genome is sequenced, and the product is then compared to a database of mutations known to be associated with drug resistance in clinical studies and/or in vitro studies. Through this comparison, a virtual phenotype for the sequence of interest is generated with predicted antiviral susceptibilities. Both Sanger sequencing and NGS platforms are available. Sanger sequencing can detect RAS where present at $\geq$15–25% of the total virus population sampled. Most NGS assays use massive parallel sequencing of short fragments, which together encompass the whole HCV genome. The large number of sequencing products is aligned to a reference genome, and a software is used to identify RAS. Minor variants present at a frequency of 1% of the total virus population sampled may be reliably detected and quantified on this platform. For clinical purposes, however, a cutoff of 15% is generally recommended [52, 53], which approximates to the results of Sanger sequencing, and below which HCV RAS are less likely to be clinically significant. Clonal sequencing can also achieve a broad characterisation of the viral quasispecies; however, it is expensive and labour-intensive and not suitable for routine clinical use.

4.4.4.3 When to Perform Resistance Testing

The barrier to resistance is a key determinant of drug potency and informs the optimal composition of a combination treatment regimen. However, multiple factors influence how easily HCV can escape from a certain regimen and the impact of resistance on treatment outcomes (Fig. 4.6). Thus, guidance on how to best employ resistance testing, if at all, to guide antiviral therapy continues to evolve. Of the three drug classes, resistance to NS5A inhibitors is clinically the most important [83]. This

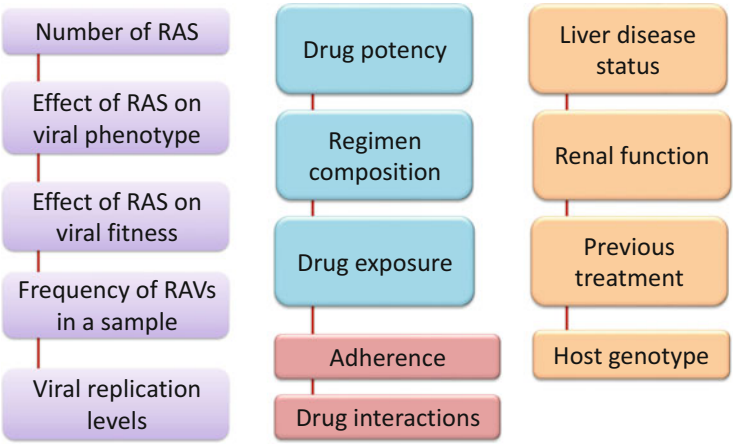


Fig. 4.6 Factors that modulate the emergence and impact of drug resistant variants in the treatment of HCV infection

reflects the substantial impact of NS5A RAS on drug susceptibility, the high fitness of NS5A resistant variants, and the ability of such variants to persist for years in the absence of drug pressure. The presence of baseline NS5A RAS has been shown to predict treatment failure of some regimens, and testing is indicated in certain circumstances before starting antiviral therapy with NS5A inhibitors [52, 53]. For example, testing is recommended in patients with HCV genotype 1a starting elbasvir/grazoprevir, and patients with elbasvir RAS should receive an alternative regimen, or longer (16 weeks) therapy with ribavirin. Although baseline NS5A RAS may impact SVR rates in a small number of patients, testing is not routinely recommended prior to starting therapy with elbasvir/grazoprevir in genotype 1b and genotype 4. Baseline NS5A RAS testing is also recommended in genotype 3-infected individuals with cirrhosis and in individuals who have previously had unsuccessful therapy with a non-NS5A inhibitor-containing regimen, both with and without cirrhosis, who are being considered for 12 weeks of sofosbuvir/velpatasvir. If Y93H is identified, addition of ribavirin, extension of therapy from 12 to 24 weeks, or an alternative regimen is recommended [52, 53].

In cases of treatment failure, the results of resistance testing may inform the choice of the retreatment regimen although guidance on interpretation of results is currently limited. Guidelines consider testing optional, although where available it should be performed to understand the patient's profile and gain knowledge of HCV resistance patterns. If testing is performed, this should be probably done immediately prior to the planned retreatment start date. This considers the possibility that any resistant variants identified months to years prior to the retreatment start date may be replaced over time by drug-susceptible variants and may not necessarily reflect the subsequent virus population. It is regarded as good practice to store a pre-treatment blood specimen (serum or plasma) for a minimum of 6 months, which if necessary may be tested retrospectively in parallel to the failure specimen to aid with the interpretation of results. The cutoff for HCV RNA level in the sample selected for

resistance testing varies according to the assay. Whilst some assays will use a lower limit of 100–300 IU/mL, others may require an HCV RNA level of at least 1000 IU/mL. The cutoff should therefore be confirmed with the testing laboratory. Suitable stored samples can be tested without the need to recall individuals.

4.4.4.4 Interpretation of Resistance Test Results

As the potential impact of RAS on predicted response to antiviral therapy varies according to multiple factors, treatment decisions are not made solely on the basis of resistance testing. It is recommended that such decisions are made by a multidisciplinary team, which is likely to include hepatologists, infectious disease physicians, pharmacists, clinical nurse specialists, and virologists. Where clinically relevant RAS are identified, consequent actions may involve one or more of:

- Avoidance of a particular regimen
- Prolongation of antiviral therapy
- Intensification of therapy with an additional drug

Newly approved regimens show improved potency and resistance barrier and may provide an effective retreatment option regardless of resistance [83]. There remain areas of uncertainty however. Further data are needed to understand (a) the impact of baseline NS5A RAS in patients with rarer genotypes and subtypes (including 1l, 4r, 3b, 3g, 6u, and 6v and some undetermined subtypes); (b) how to personalise the use of resistance testing based on individual risk factors for non-response; and (c) the role of resistance testing in guiding retreatment after treatment failure, especially in genotype 3, according to the time elapsed between first and second regimens, and in patients with decompensated cirrhosis and severe renal impairment.

4.5 Conclusions

As hepatitis C is now curable, it is imperative for the diagnostic laboratory to engage fully in efforts to increase availability of testing and to streamline the diagnostic and monitoring process. The desire for simplified algorithms must be balanced with the need to maintain quality of assessment and care, and it is the remit of the laboratory to support the development and validation of emerging diagnostic tools and methods, working alongside care providers both within and outside traditional healthcare facilities, together making HCV eradication a realistic possibility.

References

1. Feinstone SM, Kapikian AZ, Purcell RH, Alter HJ, Holland PV. Transfusion-associated hepatitis not due to viral hepatitis type A or B. N Engl J Med. 1975;292:767–70.
2. Houghton M. The long and winding road leading to the identification of the hepatitis C virus. J Hepatol. 2009;51:939–48.

3. Kuo G, Choo Q-L, Alter HJ, Gitnick GL, Redeker AG, et al. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. Science. 1989;244:362–4.
4. Kamili S, Drobeniuc J, Araujo AC, Hayden TM. Laboratory diagnostics for hepatitis C virus infection. Clin Infect Dis. 2012;55(Suppl 1):S43–8.
5. Reyes-Méndez MÁ, Juárez-Figueroa L, Iracheta-Hernández P, Medina-Islas Y, Ruiz-González V. Comparison of two diagnostic algorithms for the identification of patients with HCV viremia using a new HCV antigen test. Ann Hepatol. 2014;13:337–42.
6. Chapko MK, Dufour DR, Hatia RI, Drobeniuc J, Ward JW, Teo CG. Cost-effectiveness of strategies for testing current hepatitis C virus infection. Hepatology. 2015;62:1396–404.
7. Nastouli E, Thomson EC, Karayiannis P, Main J, McClure M, Muir D. Diagnosing acute hepatitis C in HIV-infected patients: nucleic acid testing compared with antibody and antigen-antibody detecting methods. J Clin Virol. 2009;44:78–80.
8. Baath L, Widell A, Nordenfelt E. A comparison between one first generation and three second generation anti-HCV ELISAs: an investigation in high- and low-risk subjects in correlation with recombinant immunoblot assay and polymerase chain reaction. J Virol Methods. 1992;40:287–96.
9. Colin C, Lanoir D, Touzet S, Meyaud-Kraemer L, Bailly F, Trepo C. Sensitivity and specificity of third-generation hepatitis C virus antibody detection assays: an analysis of the literature. J Viral Hepat. 2001;8:87–95.
10. Bukh J, Wantzin E, Krogsgaard K, Knudsen F, Purcell RH, Miller RH, et al. High prevalence of hepatitis C virus (HCV) RNA in dialysis patients: failure of commercially available antibody tests to identify a significant number of patients with HCV infection. J Infect Dis. 1993;168:1343–8.
11. Parry JV, Easterbrook P, Sands AR. One or two serological assay testing strategy for diagnosis of HBV and HCV infection? The use of predictive modelling. BMC Infect Dis. 2017;17(Suppl 1):705.
12. King S, Adjei-Asante K, Appiah L, Adinku D, Beloukas A, Atkins M, et al. Antibody screening tests variably overestimate the prevalence of hepatitis C virus infection among HIV-infected adults in Ghana. J Viral Hepat. 2015;22:461–8.
13. Agha S, El-Mashad N, El-Malky M, et al. Prevalence of low positive anti-HCV antibodies in blood donors: schistosoma mansoni co- infection and possible role of autoantibodies. Microbiol Immunol. 2006;50:447–52.
14. Mullis CE, Laeyendecker O, Reynolds SJ, et al. High frequency of false-positive hepatitis C virus enzyme-linked immunosorbent assay in Rakai, Uganda. Clin Infect Dis. 2013;57:1747–50.
15. Laperche S, Nübling CM, Stramer SL, Brojer E, Grabarczyk P, Yoshizawa H, et al. Sensitivity of hepatitis C virus core antigen and antibody combination assays in a global panel of window period samples. Transfusion. 2015;55:2489–98.
16. Quinti I, Hassan N, Salman D, Shalaby H, El Zimatty D, Monier MK, Arthur RR. Hepatitis C virus-specific B cell activation: IgG and IgM detection in acute and chronic hepatitis C. J Hepatol. 1995;23:640–7.
17. Coppola N, Pisapia R, Tonziello G, Masiello A, Martini S, Pisaturo M, et al. Improvement in the aetiological diagnosis of acute hepatitis C: a diagnostic protocol based on the anti-HCV-IgM titre and IgG avidity index. J Clin Virol. 2009;46:222–9.
18. Baleriola C, Johal H, Jacka B, Chaverot S, Bowden S, Lacey S, et al. Stability of hepatitis C virus, HIV, and hepatitis B virus nucleic acids in plasma samples after long-term storage at –20 degrees C and –70 degrees C. J Clin Microbiol. 2011;49:3163–7.
19. Ticehurst JR, Hamzeh FM, Thomas DL. Factors affecting serum concentrations of hepatitis C virus (HCV) RNA in HCV genotype 1-infected patients with chronic hepatitis. J Clin Microbiol. 2007;45:2426–33.
20. World Health Organization. Guidelines on Hepatitis B and C testing. Diagnostics for testing for hepatitis B and C infection. Geneva: WHO; 2017. p. 30–4.

21. Vermehren J, Kau A, Gartner BC, Gobel R, Zeuzem S, Sarrazin C. Differences between two real-time PCR-based hepatitis C virus (HCV) assays (RealTime HCV and Cobas AmpliPrep/Cobas TaqMan) and one signal amplification assay (Versant HCV RNA 3.0) for RNA detection and quantification. J Clin Microbiol. 2008;46:3880–91.
22. Chevaliez S, Bouvier-Alias M, Brillet R, Pawlotsky JM. Overestimation and underestimation of hepatitis C virus RNA levels in a widely used real-time polymerase chain reaction-based method. Hepatology. 2007;46:22–31.
23. Dubuisson J, Cosset FL. Virology and cell biology of the hepatitis C virus life cycle: an update. J Hepatol. 2014;61:S3–S13.
24. Orito E, Mizokami M, Tanaka T, Lau JY, Suzuki K, Yamauchi M, et al. Quantification of serum hepatitis C virus core protein level in patients chronically infected with different hepatitis C virus genotypes. Gut. 1996;39:876–80.
25. Chevaliez S, Soulier A, Poiteau L, Bouvier-Alias M, Pawlotsky JM. Clinical utility of hepatitis C virus core antigen quantification in patients with chronic hepatitis C. J Clin Virol. 2014;61:145–8.
26. Cresswell FV, Fisher M, Hughes DJ, Shaw SG, Homer G, Hassan-Ibrahim MO. Hepatitis C core antigen testing: a reliable, quick, and potentially cost-effective alternative to hepatitis C polymerase chain reaction in diagnosing acute hepatitis C virus infection. Clin Infect Dis. 2015;60:263–6.
27. Freiman JM, Tran TM, Schumacher SG, White LF, Ongarello S, Cohn J, Easterbrook PJ, Linas BP, Denkinger CM. Hepatitis C core antigen testing for diagnosis of hepatitis C virus infection: a systematic review and meta-analysis. Ann Intern Med. 2016;165:345–55.
28. Khan H, Hill A, Main J, Brown A, Cooke G. Can hepatitis C virus antigen testing replace ribonucleic acid polymerase chain reaction analysis for detecting hepatitis C virus? A systematic review. Open Forum Infect Dis. 2017;4:ofw252.
29. Fourati S, Feld JJ, Chevaliez S, Luhmann N. Approaches for simplified HCV diagnostic algorithms. J Int AIDS Soc. 2018;21:e25058.
30. Duchesne L, Njouom R, Lissock F, Tamko-Mella GF, Rallier S, Poiteau L, et al. HCV Ag quantification as a one-step procedure in diagnosing chronic hepatitis C infection in Cameroon: the ANRS 12336 study. J Int AIDS Soc. 2017;20:21446.
31. World Health Organization. Global hepatitis report, 2017. http://www.who.int/hepatitis/publications/global-hepatitis-report2017/en/. Accessed 28 Mar 2018.
32. Smith BD, Morgan RL, Beckett GA, Centers for Disease Control and Prevention. Recommendations for the identification of chronic hepatitis C virus infection among persons born during 1945–1965. MMWR Recomm Rep. 2012;61(RR-4):1–32.
33. Yartel AK, Rein DB, Brown KA, Krauskopf K, Massoud OI, Jordan C, et al. Hepatitis C virus testing for case identification in persons born during 1945–1965: results from three randomized controlled trials. Hepatology. 2018;67:524–33.
34. Barocas JA, Tasillo A, Eftekhari Yazdi G, Wang J, Vellozzi C, Hariri S, et al. Population level outcomes and cost-effectiveness of expanding the recommendation for age-based hepatitis C testing in the United States. Clin Infect Dis. 2018;67:549–56.
35. Torian LV, Felsen UR, Xia Q, Laraque F, Rude EJ, Rose H, et al. Undiagnosed HIV and HCV infection in a New York City Emergency Department, 2015. Am J Public Health. 2018;108:652–8.
36. Parry S, Bundle N, Ullah S, Foster GR, Ahmad K, Tong CYW, Balasegaram S, Orkin C. Implementing routine blood-borne virus testing for HCV, HBV and HIV at a London Emergency Department—uncovering the iceberg? Epidemiol Infect. 2018;17:1–10.
37. Grebely J, Applegate TL, Cunningham P, Feld JJ. Hepatitis C point-of-care diagnostics: in search of a single visit diagnosis. Expert Rev Mol Diagn. 2017;17:1109–15.
38. Greenman J, Roberts T, Cohn J, Messac L. Dried blood spot in the genotyping, quantification and storage of HCV RNA: a systematic literature review. J Viral Hepat. 2014;22:353–61.

39. Lange B, Roberts T, Cohn J, Greenman J, Camp J, Ishizaki A, et al. Diagnostic accuracy of detection and quantification of HBV-DNA and HCV-RNA using dried blood spot (DBS) samples—a systematic review and meta-analysis. BMC Infect Dis. 2017;17(Suppl 1):693.
40. Geretti AM, King S, Adjei-Asante K, Appiah LT, Owusu DO, Sarfo FS, et al. Hepatitis C virus (HCV) RNA screening and sequencing using dry plasma spots. J Clin Virol. 2017;97:18–21.
41. Mohamed Z, Mbwambo J, Shimakawa Y, Poiteau L, Chevaliez S, Pawlotsky JM, et al. Clinical utility of HCV core antigen detection and quantification using serum samples and dried blood spots in people who inject drugs in Dar-es-Salaam, Tanzania. J Int AIDS Soc. 2017;20:21856.
42. Assoumou SA, Tasillo A, Leff JA, Schackman BR, Drainoni ML, Horsburgh CR, et al. Cost-effectiveness of one-time hepatitis C screening strategies among adolescents and young adults in primary care settings. Clin Infect Dis. 2018;66:376–84.
43. Barocas JA, Linas BP, Kim AY, et al. Acceptability of rapid point-of-care hepatitis C tests among people who inject drugs and utilize syringe-exchange programs. Open Forum Infect Dis. 2016;3:ofw075.
44. Pallarés C, Carvalho-Gomes Â, Hontangas V, Conde I, Di Maira T, Aguilera V, et al. Performance of the OraQuick Hepatitis C virus antibody test in oral fluid and fingerstick blood before and after treatment-induced viral clearance. J Clin Virol. 2018;102:77–83.
45. Nash M, Huddart S, Badar S, Baliga S, Saravu K, Pai M. Performance of the Xpert® HIV-1 Viral Load assay: a systematic review and meta-analysis. J Clin Microbiol. 2018;56:e01673-17.
46. Grebely J, Lamoury FMJ, Hajarizadeh B, Mowat Y, Marshall AD, Bajis S, et al. Evaluation of the Xpert HCV Viral Load point-of-care assay from venepuncture-collected and finger-stick capillary whole-blood samples: a cohort study. Lancet Gastroenterol Hepatol. 2017;2:514–20.
47. Lamoury FMJ, Bajis S, Hajarizadeh B, Marshall AD, Martinello M, et al. Evaluation of the Xpert HCV Viral Load fingerstick point-of-care assay. J Infect Dis. 2018;217:1889–96.
48. Llibre A, Shimakawa Y, Mottez E, Ainsworth S, Buivan TP, Firth R, et al. Development and clinical validation of the Genedrive point-of-care test for qualitative detection of hepatitis C virus. Gut. 2018;67:2017–24.
49. Barreiro P, Labarga P, Fernandez-Montero JV, Poveda E, de Mendoza C, Sanchez C, et al. Longitudinal changes in viral RNA concentration in patients with chronic hepatitis C and/or HIV infection in the absence of antiviral therapy. J Clin Virol. 2013;58:391–5.
50. Zeuzem S, Ghalib R, Reddy KR, Pockros PJ, Ben Ari Z, Zhao Y, et al. Grazoprevir-elbasvir combination therapy for treatment-naive cirrhotic and noncirrhotic patients with chronic hepatitis C virus genotype 1, 4, or 6 infection: a randomized trial. Ann Intern Med. 2015;163:1–13.
51. Wyles DL, Ruane PJ, Sulkowski MS, Dieterich D, Luetkemeyer A, Morgan TR, et al. Daclatasvir plus sofosbuvir for HCV in patients coinfected with HIV-1. N Engl J Med. 2015;373:714–25.
52. American Association for the Study of Liver Diseases and the Infectious Diseases Society of America. HCV Guidance: recommendations for testing, managing, and treating hepatitis C. 2017. https://www.hcvguidelines.org/sites/default/files/full-guidance-pdf/HCVGuidance_September_21_2017_h.pdf. Accessed 10 May 2018.
53. European Association for the Study of the Liver. Recommendations on treatment of hepatitis C 2018. http://www.easl.eu/medias/cpg/2018/EASL%20Recommendations%20on%20Treatment%20of%20Hepatitis%20C%202018/English-report.pdf. Accessed 10 May 2018.
54. Sidharthan S, Kohli A, Sims Z, Nelson A, Osinusi A, Masur H, Kottilil S. Utility of hepatitis C viral load monitoring on direct-acting anti-viral therapy. Clin Infect Dis. 2015;60:1743–51.
55. Harrington PR, Deming DJ, Komatsu TE, Naeger LK. Hepatitis C virus RNA levels during interferon-free combination direct-acting antiviral treatment in registrational trials. Clin Infect Dis. 2015;61:666–7.
56. Tedder RS, Tuke P, Wallis N, Wright M, Nicholson L, Grant PR. Therapy-induced clearance of HCV core antigen from plasma predicts an end of treatment viral response. J Viral Hepat. 2013;20:65–71.

57. Loggi E, Galli S, Vitale G, Di Donato R, Vukotic R, Grandini E, et al. Monitoring the treatment of hepatitis C with directly acting antivirals by serological and molecular methods. PLoS One. 2017;12:e0187755.
58. European Union HCV Collaborators. Hepatitis C virus prevalence and level of intervention required to achieve the WHO targets for elimination in the European Union by 2030: a modelling study. Lancet Gastroenterol Hepatol. 2017;2:325–36.
59. Jülicher P, Galli C. Identifying cost-effective screening algorithms for active hepatitis C virus infections in a high prevalence setting. J Med Econ. 2018;21:1–10.
60. Smith DB, Bukh J, Kuiken C, Muerhoff AS, Rice CM, Stapleton JT, Simmonds P. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment Web resource. Hepatology. 2014;59:318–27.
61. McNaughton AL, Sreenu VB, Wilkie G, Gunson R, Templeton K, Leitch ECM. Prevalence of mixed genotype hepatitis C virus infections in the UK as determined by genotype-specific PCR and deep sequencing. J Viral Hepat. 2018;25:524–34.
62. Pham ST, Bull RA, Bennett JM, Rawlinson WD, Dore GJ, Lloyd AR, White PA. Frequent multiple hepatitis C virus infections among injection drug users in a prison setting. Hepatology. 2010;52:1564–72.
63. Hézode C. Pan-genotypic treatment regimens for hepatitis C virus: advantages and disadvantages in high- and low-income regions. J Viral Hepat. 2017;24:92–101.
64. Verbeeck J, Stanley MJ, Shieh J, Celis L, Huyck E, Wollants E, et al. Evaluation of versant hepatitis C virus genotype assay (LiPA) 2.0. J Clin Microbiol. 2008;46:1901–6.
65. Germer JJ, Majewski DW, Rosser M, Thompson A, Mitchell PS, Smith TF, et al. Evaluation of the TRUGENE HCV 5'NC genotyping kit with the new genelibrarian module 3.1.2 for genotyping of hepatitis C virus from clinical specimens. J Clin Microbiol. 2003;41:4855–7.
66. Michelin BDA, Muller Z, Stelzl E, Marth E, Kessler HH. Evaluation of the Abbott RealTime HCV assay for quantitative detection of hepatitis C virus RNA. J Clin Virol. 2007;38:96–100.
67. Vermehren J, Stelzl E, Maasoumy B, Michel-Treil V, Berkowski C, Marins EG, et al. Multicenter comparison study of both analytical and clinical performance across 4 Roche HCV RNA assays utilizing different platforms. J Clin Microbiol. 2017;55:1131–9.
68. Guelfo JR, Macias J, Neukam K, Di Lello FA, Mira JA, Merchante N, et al. Reassessment of genotype 1 hepatitis C virus subtype misclassification by LiPA 2.0: implications for direct-acting antiviral treatment. J Clin Microbiol. 2014;52:4027–9.
69. Chueca N, Rivadulla I, Lovatti R, Reina G, Blanco A, Fernandez-Caballero JA, et al. Using NS5B sequencing for hepatitis C virus genotyping reveals discordances with commercial platforms. PLoS One. 2016;11:e0153754.
70. Mourez T, Decroos A, Goria O, Montialoux H, De Oliveira F, Larrat S, Plantier JC, Riachi G. Misidentification of recombinant hepatitis C virus leading to treatment failure with direct acting antivirals. J Med Virol. 2018;90:994–7.
71. Ceccherini Silberstein F, Di Maio VC, Aragri M, Ciotti M, Cento V, Perno CF. Hepatitis C virus gene sequencing as a tool for precise genotyping in the era of new direct antiviral agents. Hepatology. 2016;63:1058–9.
72. Susser S, Dietz J, Schlevogt B, Zuckerman E, Barak M, Piazzolla V, et al. Origin, prevalence and response to therapy of hepatitis C virus genotype 2k/1b chimeras. J Hepatol. 2017;67:680–6.
73. Rodriguez C, Soulier A, Demontant V, Poiteau L, Mercier-Darty M, Bouvier-Alias M, et al. A novel standardized deep sequencing-based assay for hepatitis C virus genotype determination. Sci Rep. 2018;8:4180.
74. Sorbo MC, Cento V, Di Maio VC, Howe AYM, Garcia F, Perno CF, Ceccherini-Silberstein F. Hepatitis C virus drug resistance associated substitutions and their clinical relevance: update 2018. Drug Resist Updat. 2018;37:17–39.
75. Ribeiro RM, Li H, Wang S, Stoddard MB, Learn GH, Korber BT, et al. Quantifying the diversification of hepatitis C virus (HCV) during primary infection: estimates of the in vivo mutation rate. PLoS Pathog. 2012;8:e1002881.

76. Bagaglio S, Uberti-Foppa C, Morsica G. Resistance mechanisms in hepatitis C virus: implications for direct-acting antiviral use. Drugs. 2017;77:1043–55.
77. Jones BR, Howe AYM, Harrigan PR, Joy JB. The global origins of resistance-associated variants in the non-structural proteins 5A and 5B of the hepatitis C virus. Virus Evol. 2018;4: vex041.
78. Smith D, Magri A, Bonsall D, Ip CLC, Trebes A, Brown A, et al. Resistance analysis of genotype 3 hepatitis C virus indicates subtypes inherently resistant to nonstructural protein 5A inhibitors. Hepatology. 2019;69:1861–72.
79. Lontok E, Harrington P, Howe A, Kieffer T, Lennerstrand J, Lenz O, et al. Hepatitis C virus drug resistance-associated substitutions: state of the art summary. Hepatology. 2015;62:1623–32.
80. Palanisamy N, Kalaghatgi P, Akaberi D, Lundkvist Å, Chen ZW, Hu P, Lennerstrand J. Worldwide prevalence of baseline resistance-associated polymorphisms and resistance mutations in HCV against current direct-acting antivirals. Antivir Ther. 2018;23:485–93.
81. Dietz J, Susser S, Vermehren J, Peiffer KH, Grammatikos G, Berger A, et al. Patterns of resistance-associated substitutions in patients with chronic HCV infection following treatment with direct-acting antivirals. Gastroenterology. 2018;154:976–88.
82. Kan H, Imamura M, Uchida T, Hiraga N, Hayes CN, Tsuge M, et al. Protease inhibitor resistance remains even after mutant strains become undetectable by deep sequencing. J Infect Dis. 2016;214:1687–94.
83. Wyles DL. Resistance to DAAs: when to look and when it matters. Curr HIV/AIDS Rep. 2017;14:229–37.
84. Gane EJ, Metivier S, Nahass R, Ryan M, Stedman CA, Svarovskaia ES, et al. The emergence of NS5B resistance associated substitution S282T after sofosbuvir-based treatment. Hepatol Commun. 2017;1:538–49.
85. Bartlett SR, Grebely J, Eltahla AA, Reeves JD, Howe AYM, Miller V, et al. Sequencing of hepatitis C virus for detection of resistance to direct-acting antiviral therapy: a systematic review. Hepatol Commun. 2017;1:379–90.

Global HCV Burden

5

Angelos Hatzakis

5.1 Introduction

HCV infection and its chronic sequelae is a public health threat of international concern. Major advances in the treatment of chronic hepatitis C (CHC) led the 69th World Health Assembly (2016) to approve the Global Health Sector Strategy (GHSS) to eliminate hepatitis C (and B) infections by 2030. To achieve this goal, assessment and monitoring of global, regional, and national HCV burden during the years is a prerequisite.

The burden of HCV is represented by the burden of infection and burden of disease (Table 5.1). In each case, burden of infection is the incidence and prevalence of HCV infection and the burden of disease, the incidence and prevalence of acute and chronic hepatitis C and their clinical consequences such as chronic hepatitis C, cirrhosis, hepatocellular carcinoma (HCC), and HCV-related deaths.

Due to high rates of subclinical infection and the long latency of HCV without specific symptoms and signs, the surveillance of HCV is challenging and frequently based on statistical modeling [1].

5.2 Global Burden of HCV Infection

Using model-based meta-analysis, the Global Burden of Disease, Injuries, and Risk Factors Group estimated a >185 million of anti-HCV infected globally in 2005, representing an anti-HCV prevalence of 2.8% (95% UI 2.6–3.1) [2]. The global anti-HCV-infected population was updated in 2014 to 115 million including 104 million adults and 11 million children [3].

A. Hatzakis (✉)
Department of Hygiene, Epidemiology and Medical Statistics, National and Kapodistrian University of Athens, Medical School, Athens, Greece
e-mail: ahatzak@med.uoa.gr

A. Hatzakis (ed.), *Hepatitis C: Epidemiology, Prevention and Elimination*,
https://doi.org/10.1007/978-3-030-64649-3_5

Table 5.1 Hepatitis B and C epidemic profile: burden and response

Burden of infection (who, where, when)
Incidence: new hepatitis infections per year
Prevalence: number of hepatitis infection at a specific time point
Burden of disease (who, where, when)
Acute hepatitis cases or deaths (per year)
Chronic hepatitis C (CHC), cirrhosis, HCC cases, or deaths (per year)
Response (efficacy, coverage, effectiveness)
Prevention services (testing of blood, control of hospital infections, harm reduction, etc.)
Treatment
Monitoring of burden and response

For monitoring HCV elimination, HCV-viremia is a more meaningful marker. The Polaris Observatory HCV Collaborators, [4], using model-based meta-analysis estimated the global viremic prevalence to be 1.0% (95% UI 0.8–1.1) and the number of chronically HCV infected 71 million (62.5–79.4) The distribution of anti-HCV prevalence by country is shown in Fig. 5.1. The viremic prevalence by region is shown in Table 5.2. The highest number of viremic individuals live in Eastern Mediterranean, European, and Western Pacific Region. Genotype 1 is the most prevalent genotype (44%) followed by genotypes 3 (25%) and genotype 4 (15%). Genotype 1 is more common in high and upper middle-income countries (60%), genotype 3 is common in the lower middle-income countries (36%), and genotype 4 is common in low-income countries (45%) (Fig. 5.2) [1, 4].

5.3 Global HCV Incidence Estimates

The global HCV incidence data are provided in the WHO Global Hepatitis Report 2017 [1] (Table 5.3). The highest incidence rate and the total number of incident infections are observed in Eastern Mediterranean and European Regions, while the global estimate of incidence rate was 23.7 infections per 100.00, and the annual total number of new infections is 1,757,000.

5.4 Global HCV Burden in Specific Populations

5.4.1 Global Burden of HCV Infection Among People Who Inject Drugs (PWIDs)

The number of PWIDs globally is estimated to be 15,648,000 (10,219,000–23,737,500), representing 0.33% (0.21–0.49) of the global population (Table 5.4). The global anti-HCV prevalence among PWIDs is estimated to be 52.3% (42.4–62.1) ranging from 21.8% in Sub-Saharan Africa to 64.7% in Eastern Europe [5] (Table 5.5).

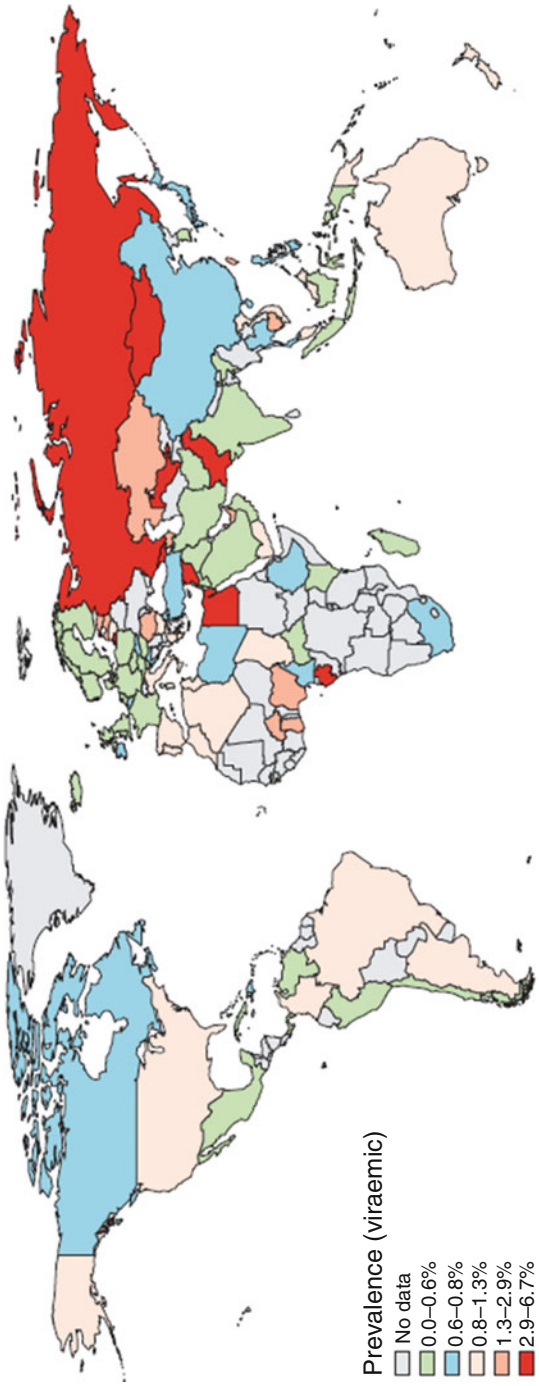


Fig. 5.1 HCV prevalence estimates [4]

Table 5.2 Global prevalence of CHC according to the WHO Global Hepatitis Report 2017 [1]

	Estimates of the prevalence of HCV infection (%)			Estimated number of persons living with HCV (millions)		
Uncertainty interval (UI)						
WHO region	Best	Lower	Higher	Best	Lower	Higher
African region	1.0	0.7	1.6	11	7	16
Region of the Americas	0.7	0.6	0.8	7	6	8
Eastern Mediterranean region	2.3	1.9	2.4	15	13	15
European region	1.5	1.2	1.5	14	11	14
Southeast Asian region	0.5	0.4	0.9	10	8	18
Western Pacific region	0.7	0.6	0.8	14	10	15
Total	1.0	0.8	1.1	71	62	79

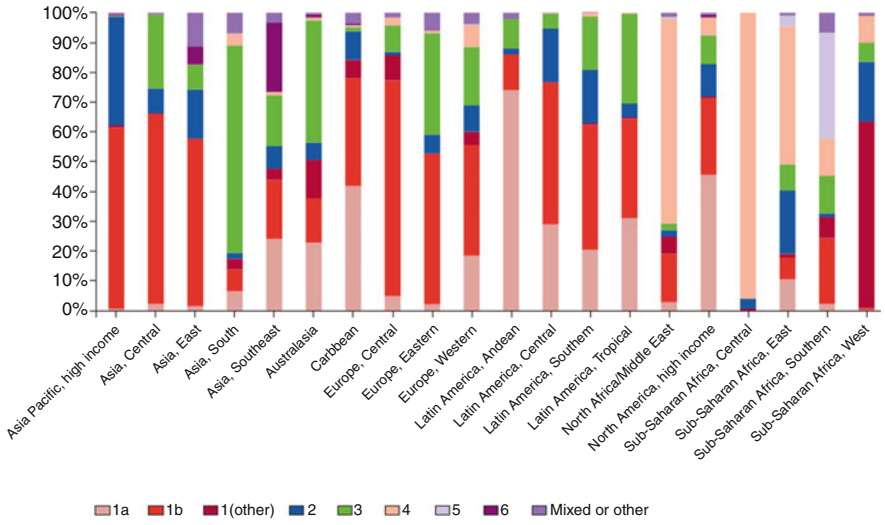


Fig. 5.2 Genotype distribution by GBC region [4]

5.4.2 Global Burden of Infection in People Coinfected with HIV and HCV (HIV/HCV Coinfected)

Data on the global prevalence of anti-HCV among HIV infected are summarized in Table 5.6. The total number of HIV/HCV coinfected is estimated to be 2.28 million (95%UI 1.271–4.417) with large HCV prevalence variation among vulnerable groups being highest in coinfected PWIDs which is 82.4% (55.2–84.5) and lowest in coinfected heterosexuals which is 4.0% (1.2–8.4) [6].

Table 5.3 Global HCV incidence according to the WHO Global Hepatitis Report 2017 [1]

Incidence of HCV infection				
	Incidence rate (per 10,000)		Total number (000)	
WHO region	Best estimate	Uncertainty interval (UI)	Best estimate	Uncertainty interval (UI)
African region	31.0	22.5–54.4	309	222–544
Region of the Americas	6.4	5.9–7.0	63	59–69
Eastern Mediterranean region	62.5	55.6–65.2	409	363–426
European region	61.8	50.3–66.0	565	460–603
Southeast Asian region	14.8	12.5–26.9	287	243–524
Western Pacific region	6.0	5.6–6.6	111	104–124
Global	23.7	21.3–28.7	1751	1572–2120

Table 5.4 Estimates of the prevalence of injecting drug use and number of people who inject drugs, by region (age 15–64 years)[a] [5]

	ALL	
	Population prevalence of IDU (%)	Estimated numbers of PWID
Eastern Europe	1.30	3,020,000
Western Europe	0.34	1,009,500
East and Southeast Asia	0.25	3,989,000
South Asia	0.09	1,023,500
Central Asia	0.63	281,500
Caribbean	0.44	79,500
Latin America	0.46	1,823,000
North America	1.06	2,557,000
Pacific Island states and territories	0.33	22,500
Australasia	0.59	115,500
Sub-Saharan Africa	0.28	1,378,000
Middle East and North Africa	0.12	349,500
Global	0.33	15,648,000

[a]For 95% UI, see [5]

5.4.3 Global Burden of Infection in Prisoners and Detainees

Among the estimated number of 10.2 million people incarcerated worldwide (2014), 1,546,500 (15.1%) have HCV infection. The prevalence of HCV in incarcerated PWIDs is high ranging from 8 to 95%. The regional prevalence of HCV among prisoners is shown in Fig. 5.3. In all regions, the prevalence is higher for HCV infection with the exception of West and Central Africa where the HBsAg prevalence prevails [7].

Table 5.5 Estimates of the anti-HCV prevalence among people who inject drugs, by region[a] [5]

	HCV	
	Prevalence among IDU (%)	Estimated number of PWID who are HCV-antibody positive
Eastern Europe	64.7	1,955,500
Western Europe	53.2	537,000
East and Southeast Asia	50.3	2,007,500
South Asia	38.6	395,000
Central Asia	54.0	152,000
Caribbean	63.6	50,500
Latin America	61.9	1,128,000
North America	55.2	1,411,000
Pacific Island states and territories	55.5	12,500
Australasia	57.1	66,500
Sub-Saharan Africa	21.8	300,000
Middle East and North Africa	48.1	168,000
Global	52.3	8,182,500

[a]For 95% UI, see [5]

Table 5.6 Global Estimates of HIV/HCV coinfection (estimate (IQR)) [6]

1. Number of HIV/HCV: 2.278 (1.271–4.417) millions
2. Number of HIV/HCV PWIDs: 1.362 (0.847–1.382) millions
3. Overall prevalence of HCV in HIV infected: 6.2 (3.4–11.9)%
4. Overall prevalence of HCV in HIV (+) general population: 2.4 (0.8–5.8)%
5. Overall prevalence of HCV in HIV (+) heterosexuals: 4.0 (1.2–8.4)%
6. Overall prevalence of HCV in HIV (+) MSM: 6.4 (3.2–10.0)%
7. Overall prevalence of HCV in HIV (+) PWIDs: 82.4 (55.2–84.5)%

5.5 Global Burden of HCV Disease

The Global Burden of Disease (GBD) Study is a systematic effort to estimate health loss due to the diseases, inquiries, and risk factors by age, sex, and geography from 1990 to 2013 including the global burden of viral hepatitis [8]. Deaths from viral hepatitis increased from 0.89 (0.86–0.94) to 1.45 million (1.38–1.54) during the years 1990 to 2013. The overall burden of deaths, years of life lost (YLLs), years living with disability (YLD), and disability-adjusted life years (DALYs) is shown in Table 5.7. Viral hepatitis is the seventh leading cause of death worldwide in 2013 compared with tenth in 1990. However, these increasing trends in DALYs are mainly due to hepatitis C (Fig. 5.4). When combined, HBV and HCV accounted for 96% (95%UI 94–97) of viral hepatitis-related mortality and 91% (88–93) of viral hepatitis-related DALYs in 2013 [8]. HBV (47%, 45–49) and HCV (48%, 46–50)

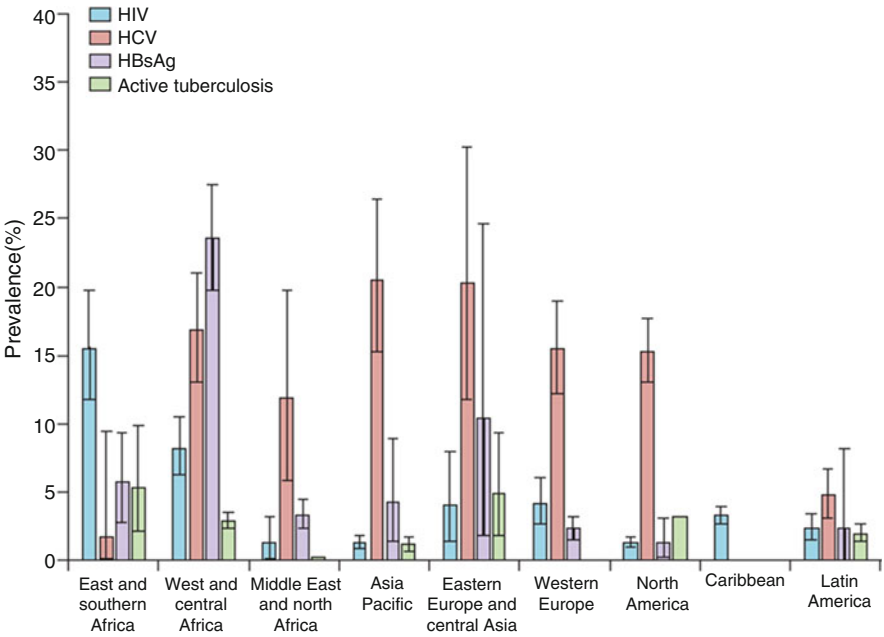


Fig. 5.3 Regional prevalence of HIV, hepatitis C, HBsAg, and active tuberculosis in prisoners, published between 2005 and 2015 [7]

Table 5.7 Deaths, YLLs, YLDs, and DALYs attributable to viral hepatitis per year[a] [8]

	Deaths, thousands	YLLs, thousands	YLDs, thousands	DALYs, thousands
1990	895	31,038	653	31,691
1995	1028	34,437	697	35,134
2000	1149	36,648	755	37,404
2005	1263	38,648	806	39,455
2010	1377	40,277	859	41,137
2013	1454	41,580	874	42,454
Percent change between 1990 and 2013	63%	34%	34%	34%

[a]For 95% UI, see [8]

were equally accounted for hepatitis-related deaths. Attributable mortality rates % due to HCV are higher in America, Europe, Middle East-Northern Africa, Oceania, and Japan (Fig. 5.5) [8]. However, the Global Health Estimates published from WHO (2015) are in disagreement with GDB in the proportion of deaths due to HBV or HCV. Of the total 1.34 million deaths due to viral hepatitis, 66% are attributed to chronic HBV and 30% to HCV [1].

HCV is a major cause of mortality and mobility among PWIDs in addition to HIV and HBV. The Global Burden of Disease Study Group estimated the global burden

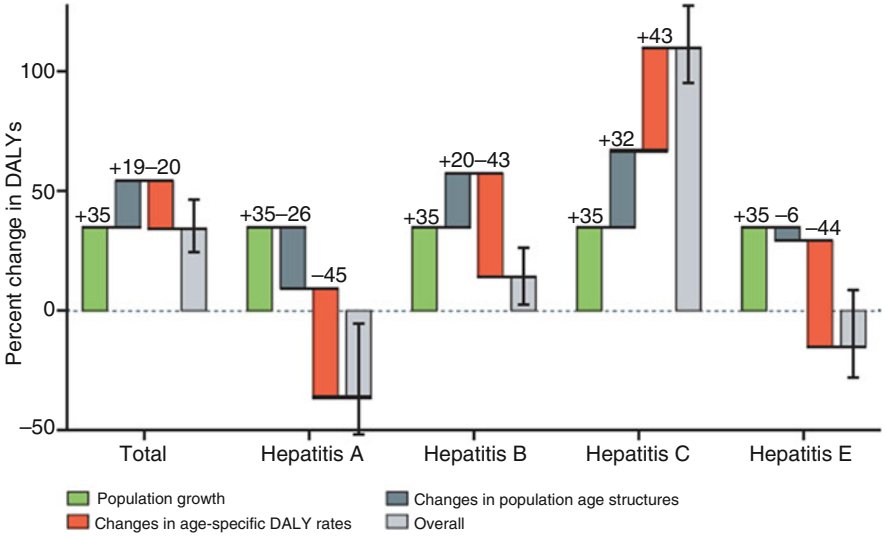


Fig. 5.4 Decomposed drivers of global changes in DALYs attributable to viral hepatitis between 1990 and 2013, by virus and for all hepatitis viruses combined [8]

of disease (YLDs, YLLs, and DALYs) attributable to intravenous drug use (IDU) as a risk factor for HIV, HBV, and HCV infection in 2013. Intravenous drug use was estimated to cause 4.0% of DALYs due to HIV, 1.1% of DALYs due to HBV, and 39.1% of DALYs due to HCV [9]. The burden of disease of HCV attributable to injecting drug use by region is shown (Table 5.8). IDU was higher in high-income North America, Southern-Latin America, Eastern Europe, Western Europe, Australasia, Central Europe, and Tropical Latin America.

5.6 Monitoring Burden of HCV Infection and Disease

The World Health Organization proposed a member of indicators to monitor HCV (and HBV) (Table 5.9) including prevalence of chronic HCV infection, people living and diagnosed with HCV, incidence of HCV, and deaths from HCC, cirrhosis, and liver diseases attributable to HCV. In addition to these burden indicators, Table 5.9 includes indicators related to prevention and treatment services. More specifically, the WHO European Region established regional targets up to 2020 for prevention and treatment services (Table 5.10) in order to achieve the HCV and HBV burden targets to reduce deaths by 20% [10].

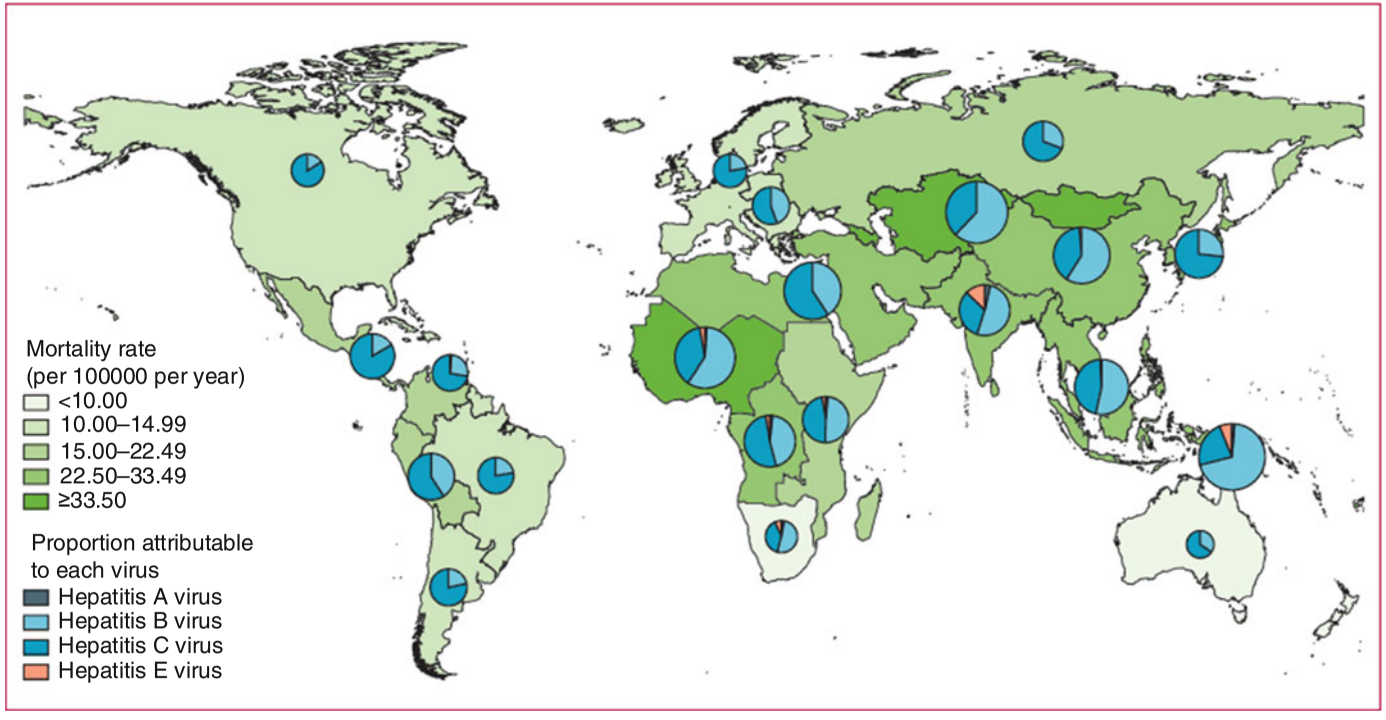


Fig. 5.5 Map of viral hepatitis-related, age-standardized mortality rate, by GBD region. Overlaid pie charts indicate each virus type's contribution to the total hepatitis-related mortality; the size of the pie charts is proportional to the region's hepatitis-attributable mortality rate. *GBD* Global Burden of Disease [8]

Table 5.8 Burden of disease of HCV attributable to injecting drug use by region in 2013[a] [8]

2013			
	Mean DALYs	Age-standardized DALY rate per 100,000	Population attributable fraction (%)
Andean Latin America	49,000	103.0	39
Australasia	21,000	59.4	59
Caribbean	32,000	73.2	38
Central Asia	73,000	91.3	25
Central Europe	153,000	97.6	52
Central Latin America	241,000	109.7	31
Central Sub-Saharan Africa	39,000	65.8	19
East Asia	2,425,000	140.5	49
Eastern Europe	605,000	231.1	68
Eastern Sub-Saharan Africa	176,000	83.8	34
High-income Asia Pacific	373,000	144.5	46
High-income North America	810,000	177.2	81
North Africa and Middle East	119,000	29.6	7
Oceania	7000	85.5	28
South Asia	216,000	14.6	7
Southeast Asia	525,000	85.4	28
Southern Latin America	112,000	171.5	70
Southern Sub-Saharan Africa	14,000	23.2	26
Tropical Latin America	268,000	126.4	54
Western Europe	705,000	120.1	64
Western Sub-Saharan Africa	84,000	37.4	11
Global	7,046,000	101.1	38

[a]For 95%UI, see [8]

5.7 Summary

The global HCV burden of infection is 115 million and the global burden of chronic hepatitis C 71 million. The global genotype distribution is HCV-1, 44%; HCV-3, 25%; and HCV-4, 15%. The highest number of infected people live in Eastern Mediterranean, European, and Western Pacific regions. The global number of

Table 5.9 Summary of indicators for monitoring and evaluation on viral hepatitis B and C [10]

Section 1. Core indicators: essential indicators to monitor and report progress at global and national levels		
Indicator number	Indicator name	Programmatic area
C.1	(a) Prevalence of chronic HBV infection	Viral hepatitis
	(b) Prevalence of chronic HCV infection	
C.2	Infrastructure for HBV and HCV testing	
C.3	(a) Coverage of timely hepatitis B vaccine birth (dose within 24 h) and other interventions to prevent mother-to-child transmission of HBV	Immunization
	(b) Coverage of third-dose hepatitis B vaccine among infants	Immunization
C.4	Facility—level injection safety	Injection safety
C.5	Needle-syringe distribution	Harm reduction
C.6	People living with HCV and/or HBV diagnosed	Viral hepatitis
C.7	(a) Treatment coverage for hepatitis B patients	
	(b) Treatment coverage for hepatitis C patients	
C.8	(a) Viral suppression for chronic hepatitis B patients treated	
	(b) Cure for chronic hepatitis C patients treated	
C.9	(a) Cumulated incidence of HBV infection in children 5 years of age	
	(b) Incidence of HCV infection	
C.10	Deaths from hepatocellular carcinoma (HCC), cirrhosis, and liver diseases attributable to HBV and HCV infection	

Table 5.10 WHO-EURO Regional Targets up to 2020 [10]

• 95% coverage with three-dose HBV vaccine for infants, in countries that implement universal childhood vaccination
• 90% coverage with interventions to prevent mother-to-child transmission of HBV (hepatitis B birth-dose vaccination or other approaches)
• 100% of blood donations screened using quality-assured method
• 50% of injections administrated with safety-engineered injection devices, integrated into broader infection prevention and control
• At least 200 sterile injection equipment kits distributed per person per year for people who inject drugs, as part of comprehensive package of harm reduction services
• 50% of people living with chronic HBV and HCV infections are diagnosed and aware of their condition
• 75% treatment coverage of people diagnosed with HBV and HCV infections who are eligible for treatment

PWIDs is 15,648,000 and 52.3% are infected by HCV. The number of HCV-/HIV-coinfected people is estimated to be 2.28 with the overall prevalence of HCV in HIV-infected individuals of 6.2%. The HCV prevalence in HCV/HIV (+) heterosexuals, MSM, and PWIDs is estimated to be 4.0%, 6.4%, and 82.4%,

respectively. The HCV prevalence among the global estimate of 10.2 million prisoners and detainees is estimated to be 15.1%.

Viral hepatitis is the seventh leading cause of death. There is no consensus in the fraction of viral hepatitis deaths causally related to HBV and HCV.

The World Health Organization in 2016 approved the Global Health Sector Strategy for Elimination of Viral Hepatitis up to 2030 and recommended burden and service indicators to monitor the progress of viral hepatitis elimination.

References

1. Global Hepatitis Report 2017, WHO.
2. Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. Hepatology. 2013;57(4):1333–42.
3. Gower E, Estes C, Blach S, Razavi-Shearer K, Razavi H. Global epidemiology and genotype distribution of the hepatitis C virus infection. J Hepatol. 2014;61(1 Suppl):S45–57.
4. The Polaris Observatory HCV Collaborators. Global prevalence and genotype distribution of hepatitis C virus infections in 2015: a modelling study. Lancet Gastroenterol Hepatol. 2017;2 (3):161–76.
5. Degenhardt L, Peacock A, Colledge S, Leung J, Grebely J, Vickerman P, Stone J, Cunningham EB, Trickey A, Dumchev K, Lynskey M, Griffiths P, Mattick RP, Hickman M, Larney S. Global prevalence of injecting drug use and sociodemographic characteristics and prevalence of HIV, HBV, and HCV in people who inject drugs: a multistage systematic review. Lancet Glob Health. 2017;5(12):e1192–207.
6. Platt L, Easterbrook P, Gower E, McDonald B, Sabin K, McGowan C, Yanny I, Razavi H, Vickerman P. Prevalence and burden of HCV co-infection in people living with HIV: a global systematic review and meta-analysis. Lancet Infect Dis. 2016;16(7):797–808.
7. Dolan K, Wirtz AL, Moazen B, Ndeffo-Mbah M, Galvani A, Kinner SA, Courtney R, McKee M, Amon JJ, Maher L, Hellard M, Beyrer C, Altice FL. Global burden of HIV, viral hepatitis, and tuberculosis in prisoners and detainees. Lancet. 2016;388(10049):1089–102.
8. Stanaway JD, Flaxman AD, Naghavi M, Fitzmaurice C, Vos T, Abubakar I, Abu-Raddad LJ, Assadi R, Bhala N, Cowie B, Forouzanfour MH, Groeger J, Hanafiah KM, Jacobsen KH, James SL, MacLachlan J, Malekzadeh R, Martin NK, Mokdad AA, Mokdad AH, Murray CJL, Plass D, Rana S, Rein DB, Richardus JH, Sanabria J, Saylan M, Shahraz S, So S, Vlassov VV, Weiderpass E, Wiersma ST, Younis M, Yu C, El Sayed Zaki M, Cooke GS. The global burden of viral hepatitis from 1990 to 2013: findings from the Global Burden of Disease Study 2013. Lancet. 2016;388(10049):1081–8. https://doi.org/10.1016/S0140-6736(16)30579-7.
9. Degenhardt L, Charlson F, Stanaway J, Larney S, Alexander LT, Hickman M, Cowie B, Hall WD, Strang J, Whiteford H, Vos T. Estimating the burden of disease attributable to injecting drug use as a risk factor for HIV, hepatitis C, and hepatitis B: findings from the Global Burden of Disease Study 2013. Lancet Infect Dis. 2016;16(12):1385–98.
10. Action Plan for the Health sector Response to viral Hepatitis in the WHO European Region, WHO.

Epidemiology of Hepatitis C Virus: People Who Inject Drugs and Other Key Populations

6

Lucas Wiessing, Isabelle Giraudon, Erika Duffell, Irene Veldhuijzen, Ruth Zimmermann, and Vivian Hope

6.1 Introduction

Hepatitis C virus (HCV) infection is a serious public health problem, with globally 71 million people estimated to be chronically infected and at risk of long-term sequelae, including liver cirrhosis and hepatocellular carcinoma [1–3]. Acute infection is typically asymptomatic, and owing to HCV's ability to evade the immune system, 70–80% of infections become chronic [4–6]. In those chronically infected, it may be decades after initial infection before significant sequelae develop. If left untreated, chronic liver disease will progress to cirrhosis in 5–20%, and 1–5% will die from decompensated cirrhosis or hepatocellular carcinoma [7]. HCV infection contributes to around 27% of liver cirrhosis cases and 25% of primary liver cancers, and resulted in an estimated 400,000 deaths worldwide from these complications in 2015 [1, 8]. Co-infections with HIV are an increasing problem in countries with HIV

L. Wiessing (✉) · I. Giraudon
European Monitoring Centre for Drugs and Drug Addiction, Lisbon, Portugal
e-mail: Lucas.Wiessing@emcdda.europa.eu; Isabelle.Giraudon@emcdda.europa.eu

E. Duffell
European Centre for Disease Prevention and Control, Stockholm, Sweden
e-mail: Erika.Duffell@ecdc.europa.eu

I. Veldhuijzen
Centre for Infectious Disease Control, National Institute for Public Health and the Environment, Bilthoven, The Netherlands
e-mail: irene.veldhuijzen@rivm.nl

R. Zimmermann
Department of Infectious Disease Epidemiology, Robert Koch Institute, Berlin, Germany
e-mail: ZimmermannR@rki.de

V. Hope
Public Health Institute, Liverpool John Moores University, Liverpool, UK
e-mail: v.d.hope@ljmu.ac.uk

A. Hatzakis (ed.), *Hepatitis C: Epidemiology, Prevention and Elimination*,
https://doi.org/10.1007/978-3-030-64649-3_6

epidemics in people who inject drugs (PWID), and among men who have sex with men (MSM), and underlying viral hepatitis is becoming a major cause of death among people with HIV [1, 9].

In May 2016, the World Health Assembly adopted the first Global Health Sector Strategy (GHSS) on viral hepatitis, aimed at eliminating hepatitis B and C as public health threats [10]. To achieve elimination, continued primary prevention efforts are needed as well as secondary prevention through screening, linkage to care and treatment of people with chronic HCV infection. There is currently no vaccine for HCV; however, research in this area is ongoing [11]. Although antiviral treatment for HCV has been available since 2001, the more recent development of highly effective direct-acting antiviral (DAA) therapy, which can cure more than 95% of persons with HCV infection, has now made elimination of HCV as a public health threat a possibility [3, 12, 13]. However, the largely asymptomatic nature of the infection until its later stages hinders early diagnosis, and access to treatment remains a problem in many countries. In Canada and the USA, it has been estimated that fewer than 5% of PWID have received treatment for chronic HCV [14, 15], while in Europe, this may be under 10% [3].

HCV is usually transmitted parenterally. Within high-income countries, HCV transmission through blood products has effectively been halted, leaving PWID as the group most affected by HCV infection [16, 17]. In these countries, HCV transmission is concentrated among PWID [18, 19], with between 50 to 80% chronically infected [3, 19]. In Europe, PWID, or people who have injected in the past, are now the main group affected [20–22]. In medium- and low-income countries, however, iatrogenic HCV transmission still accounts for a significant proportion of incident infections [23].

There are high numbers of PWID among most prison populations [24], and as a consequence, the burden of HCV is often high among prisoners, with a recent global meta-analysis estimating over one-quarter of inmates are positive for anti-HCV, equating to approximately 1.65 million with chronic HCV infection [25]. Furthermore, HCV transmission within prison is not an uncommon occurrence, often due to a lack of access to harm reduction interventions [25]. In many European countries, another population group at higher risk than the general population of having a chronic infection are migrants born in countries with high endemicity of HCV [26]. There is also increasing concern surrounding HCV among MSM, particularly those living with HIV. Although they contribute less towards the overall HCV epidemic than PWID, those who are co-infected are often in urgent need of HCV treatment and prevention interventions due to accelerated liver disease progression and increased mortality [27, 28]. Finally, there is still a significant fraction (up to 45% in some countries) of acute HCV infections for which the mode of transmission cannot be identified. Suggested explanations include undisclosed risk factors and possibly transmission by acupuncture, tattooing, piercing or shaving by barbers [29–31].

The aim of the chapter is to describe the epidemiology of HCV infection among PWID and other key risk groups and to identify knowledge gaps that are important for prevention and treatment. Given that data on specific risk groups are often scarce,

we have used both epidemiological data on HCV in general population groups and among these risk groups. Our sources have been key articles and reports already known to us, complemented by a search in PubMed on 'HCV epidemiology' limited to 'reviews' (resulting in 1623 items on August 1, 2017).

6.2 People Who Inject Drugs

6.2.1 Prevalence and Trends

A recent systematic review reported on the global prevalence of injecting drug use, the sociodemographic characteristics of PWID and prevalence of HIV, HBV and HCV in this group [32]. This review estimated that 52.3% (42.4–62.1%) of current PWID have been exposed to HCV (are anti-HCV positive), equating to 8.2 (4.7–12.4) million people. In most regions and countries, more than half of PWID have been exposed to HCV. PWID in sub-Saharan Africa had a lower prevalence of anti-HCV (21.8%, 17.6–26.5) compared with regions, such as Western Europe (53.2%, 48.4–57.9), where injecting drug use has been established for longer. High anti-HCV prevalence was estimated in some countries in east and Southeast Asia (e.g. Indonesia 89.2% (85.3–92.3), Taiwan 91.0% (89.5–92.4) and Thailand 88.5% (82.6–92.9)), although the regional estimated prevalence was lower (50.3%, 37.7–62.8), largely because HCV antibody prevalence among PWID in China (43.1%, 27.5–58.6) was estimated to be lower (Fig. 6.1) [32]. It is estimated that overall about 75% of those exposed to HCV infection will have an ongoing chronic infection and are at risk of long-term sequelae [33], although there may be sizeable variation in chronicity levels between countries and PWID populations [3].

Across Europe, the HCV antibody prevalence among PWID is high overall. A European review found the estimated anti-HCV prevalence in PWID was on average almost 50 times higher than that in the general population, in the 13 countries that had estimates of prevalence in both groups [34]. In more recent data obtained by the EMCDDA, 13 countries reported on anti-HCV prevalence among national samples of PWID for the years 2014 or 2015, with prevalence ranging from 15% to 84%, with prevalence in excess of 50% in five countries (Fig. 6.2) [36].

Monitoring of anti-HCV prevalence within populations over time provides an indication of possible changes in the transmission of the virus. Among EU countries reporting to EMCDDA with national trend data among PWID for the period 2010–2015, three observed an increase in HCV antibody prevalence, while four observed a decrease. Data at the subnational (local, regional) level are important as HCV prevalence can be very heterogeneous, and studies in Europe have shown local increases in prevalence in Budapest (Hungary), Sofia (Bulgaria) and Vienna (Austria) [37].

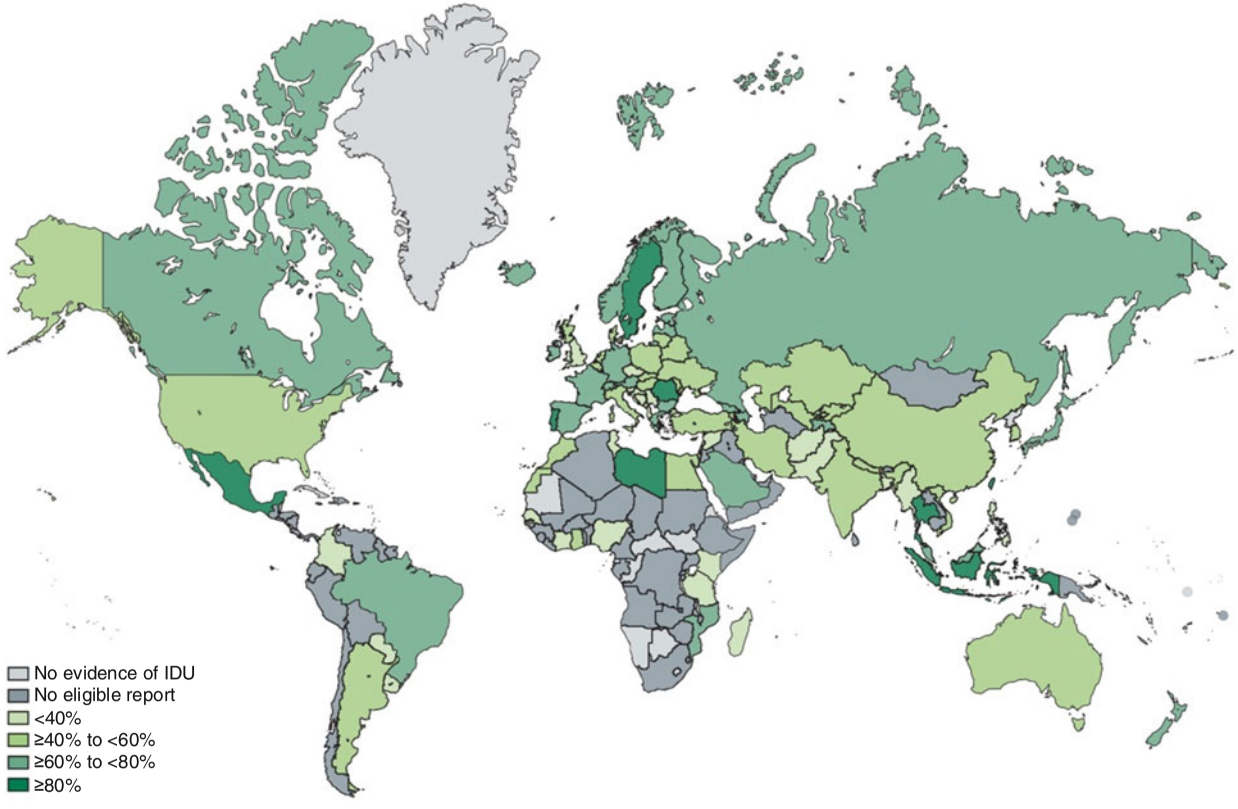


Fig. 6.1 Estimated anti-hepatitis C virus prevalence among people who inject drugs by country. Source: [32]. *IDU*: injecting drug use. No eligible report: evidence of IDU located, but no study of HCV antibody prevalence among people who inject drugs that met the eligibility criteria was located

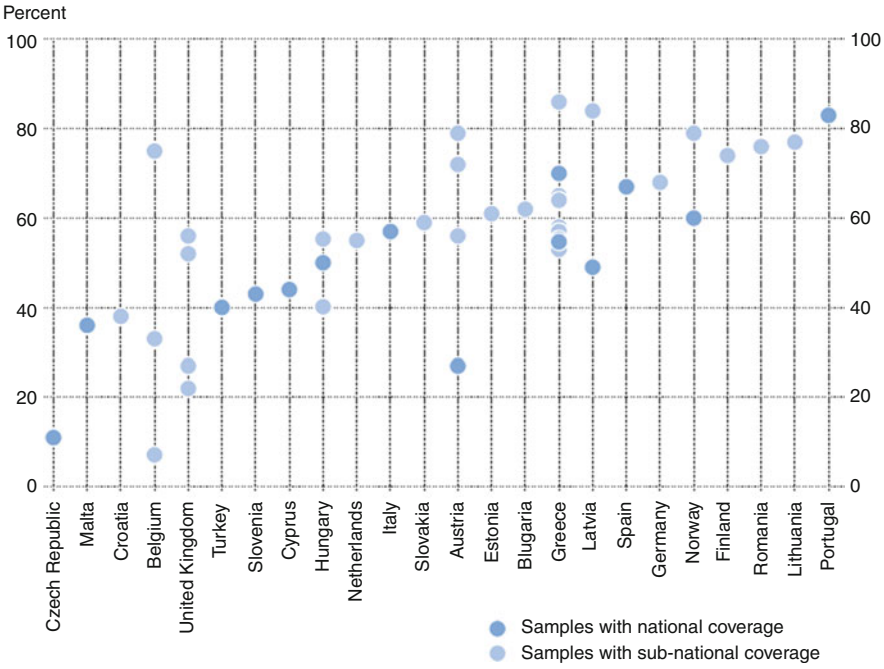


Fig. 6.2 Estimated anti-hepatitis C virus prevalence among people who inject drugs in Europe, 2014–2015. Source: [35]

6.2.2 Incidence

Incidence of new HCV infections is difficult to estimate directly as this requires studies that follow up those at risk over time; thus, globally, data on HCV incidence are not available for most countries. More recently, studies estimate incidence using biological markers of recent infection (e.g. HCV RNA in the absence of antibodies and antibody avidity) [38–44] and using indirect methods, such as back-calculations using the known number of total HCV infections in a given year and subtracting the number spontaneously cleared, cured and those who died in that year [45–47]. Globally, estimates of HCV incidence among PWID range from 5% to 45%/year [28]. As incidence in the general population is thought to mostly reflect incidence in PWID, both are discussed here.

A recent modelling study in 15 countries concluded that the overall annual incidence of HCV infection in the general population has reached its peak in most countries, although annual incidence seems to be still increasing in Russia [46]. Although surveillance data on reported diagnoses have to be interpreted with

great care, as they can reflect increases in testing and better reporting, a nationwide study from the USA for the period 2004–2014 found significant concurrent increases in reported cases of acute HCV infection and treatment admissions for injection of opioids [48]. The increase in incidence was largest in persons aged 18–29 years (400%) and 30–39 years (325%) and among non-Hispanic Whites and Hispanics. In the years 2011–2014, over 75% of the cases with acute HCV infection with risk factor data reported injecting drug use. In another study in the USA, a cohort study in five cities, the incidence of HCV infection in young PWID was 17.2 infections/100 person-years (PY) [49].

Studies reporting on the incidence of primary HCV infection among PWID in Europe have been reviewed by the EMCDDA [3]. In total, 27 studies were found that reported direct measurements of HCV incidence, covering only eight EU Member States (Czech Republic, Denmark, Finland, France, Ireland, Netherlands, Spain, Sweden) and the UK. In these studies, the incidence of HCV among PWID was often high (range 2.7–66/100 PY, median 13). The review found that studies of incidence of HCV infection among PWID were sparse across Europe, of variable quality and not easily comparable. Some of the studies had limitations such as being old, conducted in specific settings such as needle and syringe programmes or covering a small local area. While the review covered literature published from 2000 to 2012, studies published after 2005 were found only for the Netherlands and the UK [37].

As approximations of recently acquired infections or incidence, the EMCDDA monitors the prevalence of anti-HCV among young PWID (those under 25 years old) and among new PWID (those injecting for less than 2 years) among countries in Europe. Estimates for these subgroups of PWID are available only for a few countries and are often based on a small number of people. Overall, they indicate anti-HCV prevalence levels of between 20% and 60% in these groups. In common with the findings on anti-HCV prevalence among PWID of all ages and injection history, the highest estimates are among those in the south or east of Europe [37]. The latest data at the time of writing (2017) show that many of the countries reported samples where anti-HCV prevalence is 40% or more among young PWID, suggesting high levels of transmission in recent years (Fig. 6.3) [35, 36].

6.2.3 Genotypes

HCV can be classified into seven genotypes, numbered 1–7, and 67 subtypes [50]. Globally, genotype 1 (G1) is the most common (46%), followed by G3 (22%), G2 (13%) and G4 (13%) [51]. Some of the genotypes (1 and 3—in particular subtypes 1a, 1b and 3a) have become distributed widely because of transmission through blood transfusion and needle-sharing among PWID and now represent the vast majority of infections in developed countries [37, 52]. As new DAA treatments are effective across all genotypes, this variation in genotypes is now becoming less important; however, some variation in treatment success still exists by genotype [53]. Treatment regimens, duration of treatment and cure rates, as well as clinical

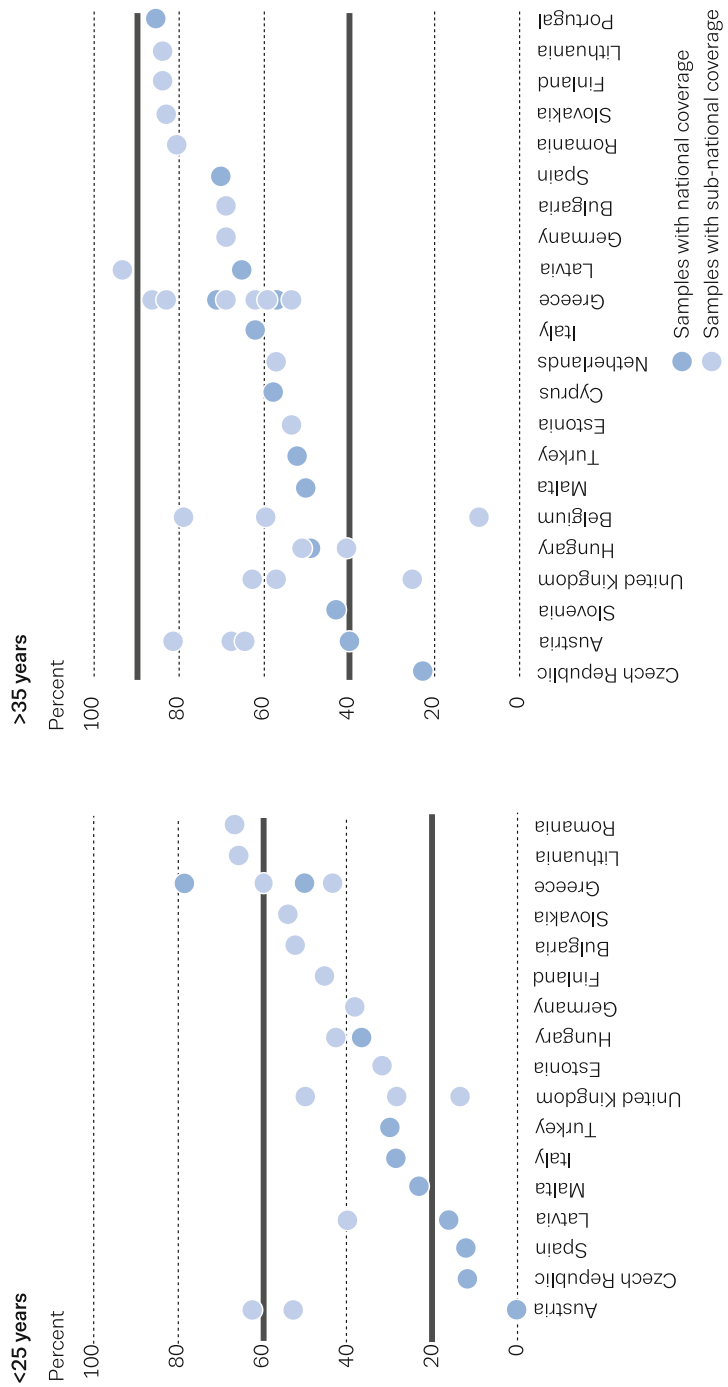


Fig. 6.3 Estimated anti-hepatitis C virus prevalence among people who inject drugs in Europe, by age; studies with national and subnational coverage, 2014–2015. Source: [35]
NB: The heavy lines in the graphics highlight the differences in the distributions of the two age groups. Studies with sample size of less than 10 are not available for all countries within artwork (similar to Fig. 6.12)

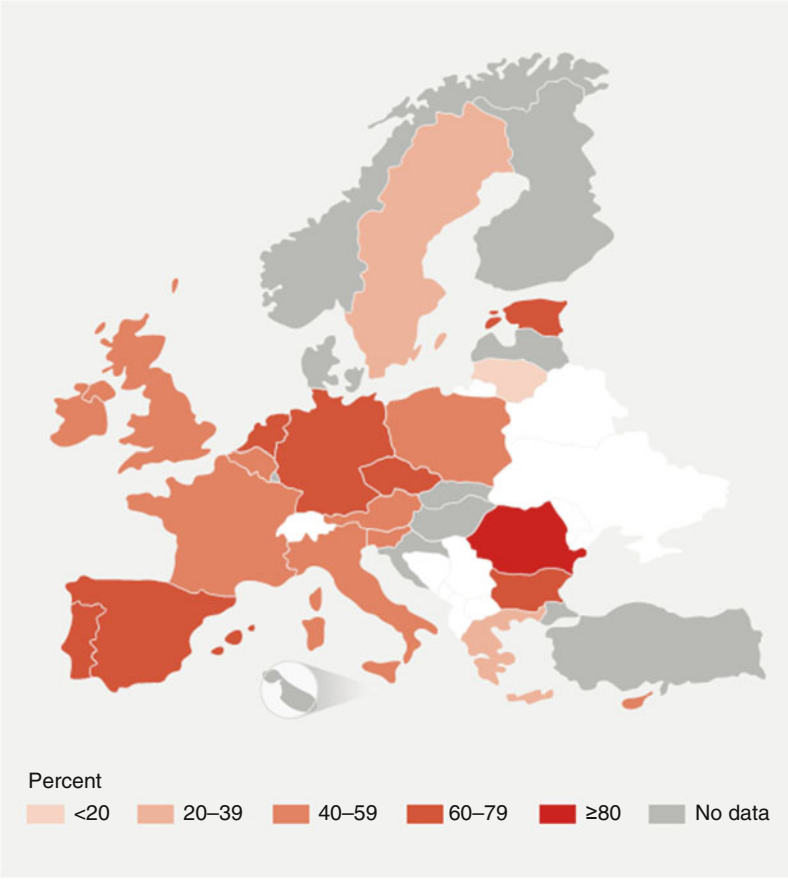


Fig. 6.4 Proportion (%) of HCV infections among people who inject drugs in Europe that are genotypes 1 or 4. Source: [3]

course, vary by genotypes, with genotype 3 being associated with an increased risk of cirrhosis and hepatocellular cancer [54, 55]. Genotyping is also an important tool to better understand the epidemiology of HCV [56].

In an EMCDDA review of HCV epidemiology among PWID in Europe [3], 36 studies with genotype data were identified from 20 EU countries, including samples for nearly 6000 HCV-infected PWID which were identified to the level of genotype or subtype. HCV genotypes 1 and 3 (subtypes 1a and 3a) are the most commonly identified among PWID in Europe. The data suggest that genotype 4, prevalent in the Middle East and Africa, particularly in Egypt [57], may be increasing. Distribution of the genotypes varied among PWID across Europe (Fig. 6.4) with genotypes 1 and 4 being predominant in certain EU countries (in particular Portugal, Romania and Spain), and showing a large variation across

the EU (prevalence of genotypes 1 or 4: 17–91%, median 53%) [3]. Caution must be exercised in interpreting these findings for a number of reasons: not all reports assessed mixed infections (to see if multiple genotypes are present); estimates for six of the countries are based on samples of fewer than 100 patients; for ten countries, only one study could be located; and some studies were based on selected populations (such as hospitalised patients) [37].

6.2.4 HIV Co-infection

Globally, it is estimated that there are approximately 2,278,400 (Interquartile range (IQR) 1,271,300—4,417,000) people living with HIV–HCV co-infections of which 59.8% (31.3—66.7%) are PWID [58]. In HIV-infected individuals, HCV co-infection is estimated at 2.4% (IQR 0.8–5.8) within general population samples, 4.0% (1.2–8.4) among pregnant women or heterosexuals, 6.4% (3.2–10.0) in MSM and 82.4% (55.2–88.5) in PWID [58]. Rates of co-infection are high in sub-Saharan Africa, with an average anti-HCV prevalence of 7% among those HIV-infected and with levels of HIV co-infection especially high among PWID [59].

A systematic review by the EMCDDA for PWID found 68 HIV–HCV co-infection estimates in Europe, among 33 published and 15 unpublished studies [3]. As HCV infection was not confirmed by RNA in many studies, antibody prevalence was used across all studies. Estimates of HIV–HCV co-infection prevalence were available for 22 countries in Europe with 11 countries having multiple estimates. Among HCV-infected PWID, co-infection with HIV ranged from 0% to 70%, with a median of 3.9%. The level of HIV-HCV co-infection correlated with the HIV prevalence. HIV prevalence among PWID differs greatly across Europe ranging from 0% to 30%. Levels of co-infection prevalence can be classed as low (not more than 4%) in 11 countries, moderate (5–15%) in three countries and high (over 15%) in seven countries (Fig. 6.5) [37].

An increase in HCV prevalence among PWID has previously been associated with an increased risk for injection-related HIV outbreaks, and therefore increases in HCV should be monitored carefully [37, 60–62].

6.2.5 Risk Factors

Sharing needle/syringes is the main route of HCV acquisition among PWID. Despite strong declines over time in some high-income countries globally, this risk behaviour generally persists among PWID [63–65]. In Europe, a similar decline has been seen in Western European countries [66–69]; however, the prevalence of sharing needles/syringes may remain high in Eastern Europe [70, 71].

The declines in needle/syringe sharing have helped reduce HIV incidence in PWID in many high-income countries, but not HCV incidence, as the shared use of other drug preparation materials persists [72, 73] and HCV is more easily transmitted than HIV [74]. The context in which PWID inject is characterised by a

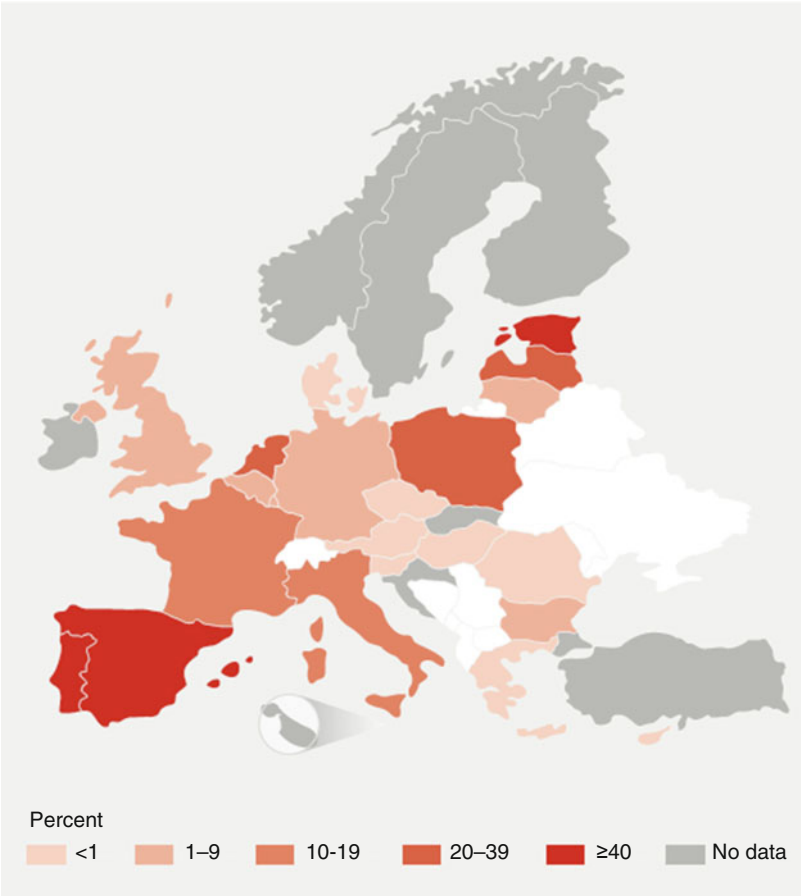


Fig. 6.5 Co-infection with HIV among HCV-infected people who inject drugs in Europe. Source: [3]

high prevalence of HCV and a wide range of injecting equipment that can transmit HCV [75]. The risk of sharing needle/syringes has been reasonably well established despite inconsistencies between individual studies. However, the discussion on the contribution of other injecting paraphernalia (e.g. cookers, filters) or behaviours (e.g. sharing drugs) and non-injecting drug paraphernalia (e.g. sniffing straws) has not yet been fully resolved.

A meta-analysis of 16 studies undertaken in Europe during 1990–2011 found that pooled prevalence and incidence of HCV were 59% and 11%, respectively, among PWID who reported never (in some studies: not recently) sharing needle/syringes. A pooled odds ratio (OR) of 3.3 (95% CI 2.4–4.6) was found, comparing HCV infection among those who ever (or recently) shared needle/syringes relative to those who reported never (or not recently) sharing. Differences were found when studies were stratified by recruitment setting (prison vs. drug treatment sites),

recruitment method (outreach vs. non-outreach), sample HCV prevalence and sample mean/median time since onset of injecting [76].

A prospective study in San Francisco in 2000–2001 found that sharing needle/syringes with an HCV-infected sex partner or a person who was not a sex partner, sharing non-sterile drug-preparation equipment, pooling money with another PWID to buy drugs, and exchanging sex for money were significantly associated with infection in young PWID [77]. In another cohort study in five cities in the USA (2002–2004), the incidence of HCV infection in young PWID was 17.2 infections/100 PY. Adjusting for confounders, the shared use of drug preparation equipment was significantly associated with HCV seroconversion (adjusted hazard ratio, 2.66; 95% confidence interval, 1.03–23.92), but needle/syringes sharing was not (adjusted hazard ratio, 0.91). It was estimated that 37% of HCV seroconversions in PWID were due to the sharing of drug preparation equipment [49]. Another analysis in a cohort of 317 PWID (1994–1997) found that among those who did not share needle/syringes, HCV seroconversion was associated with sharing drug cookers and filtration cotton (adjusted risk ratio 5.9; 95% confidence interval 1.1, 31.7); 54% of HCV infections in injection drug users who did not share needle/syringes were attributable to cooker/cotton sharing [78].

A recent experimental study suggests that the associations found in many studies with non-syringe equipment ('paraphernalia': cookers, filters) may actually reflect transmissions resulting from syringe-mediated sharing of drugs [79].

Equipment used for taking drugs by non-injecting routes might also be a risk for HCV transmission: using contaminated straws for snorting drugs might put people at risk of infection [80].

6.2.6 Disease Progression, Cirrhosis, Hepatocellular Carcinoma, Burden of Disease and Mortality

Information on the current and projected impact of HCV infection in terms of disease burden and mortality is necessary to inform public health planning and resource allocation. Burden of disease studies aim to quantify the effect of an illness in terms that are comparable across populations and between diseases. Data on the burden of disease due to HCV are however scarce, outdated or inconclusive [3, 37, 81].

Overall, an estimated 27% of liver cirrhosis cases and 25% of primary liver cancers result from HCV infection [8]. Approximately 400,000 people die each year from HCV, mostly from cirrhosis and hepatocellular carcinoma [11]. Between 1990 and 2013, global HCV deaths increased from 303,000 to 704,000 [82]. In the USA, mortality related to HCV nationally surpasses that from human immunodeficiency virus (HIV) infection [83, 84].

A systematic review of disease progression in PWID found that the pooled incidence rates of compensated cirrhosis, decompensated cirrhosis and hepatocellular carcinoma were 6.6 (95% CI 4.8, 8.4), 1.1 (95% CI 0.8, 1.4) and 0.3 (95% CI 0.1, 0.6) events/1000 PY, respectively. Average time to cirrhosis using pooled stage-constant fibrosis progression rates is 34 years post-infection, and time to METAVIR stage F3 is 26 years; using stage-specific estimates, time to cirrhosis is 46 years and

time to F3 is 38 years. Thus, left untreated, many PWID with chronic HCV infection will develop liver sequelae in mid- to late adulthood [85]. A recent cohort study in England showed that liver disease (including viral hepatitis and cirrhosis) is one of the major causes of deaths among PWID [86].

A review conducted by the EMCDDA [3] of literature published 2000–2012 found seven studies that reported on the burden of disease or mortality related to HCV infection among PWID in the European Union and the UK. Where assessed, the disease burden of HCV was found to be substantial and was expected to rise in the next decade. Only 2 of the 27 countries included in the review appeared to have carried out a modelling study to estimate the effect of HCV treatment on the future burden of disease. Without treatment, a study in the Netherlands (Amsterdam) projected a 36% increase in the occurrence of decompensated cirrhosis or hepatocellular cancer, between 2011 and 2025 [87], whereas in Scotland, UK (Glasgow), increases of 56% in cirrhosis and 64% in mild liver disease were projected for 2010–2025. Both studies showed that HCV treatment would substantially reduce the burden of liver disease [88].

Mortality in HCV-infected PWID is dependent on competing mortality (e.g. due to HIV infection or overdose) and the duration of persistent HCV infection. In the review, all-cause mortality rates among HCV-infected PWID were estimated at 2.1–2.4/100 PY in Spain [89] and the Netherlands [90], while a much higher rate was estimated for PWID co-infected with HIV in Denmark, where all-cause mortality was estimated at 12.2/100 PY [91]. The high mortality rate in this Danish study may be explained by high local rates of overdose mortality and differences in antiretroviral therapy regimes compared to the Spanish study that reported a crude mortality rate of 2.4/100 person-years among HIV-co-infected PWID during a comparable study period. This suggests the existence of significant differences between countries in mortality rates among HIV-infected PWID, as is found for mortality among all PWID, and underlines the importance of obtaining country-specific mortality estimates.

The HCV disease burden among PWID translates to a significant burden in the general population. In Europe, annual mortality rates from hepatocellular cancer vary by country and are generally lower in countries in the north-west of Europe compared with those in the south-east, possibly reflecting historic differences in risk (Fig. 6.6). The main causes of hepatocellular cancer are HBV and HCV infections and alcohol consumption. In all countries, mortality from hepatocellular cancer is higher in males than in females [92]. Although these data are not specific to PWID, they provide the scale of morbidity and mortality related to liver cancer, a large proportion of which is accounted for by chronic viral hepatitis infection acquired through injecting drugs [37].

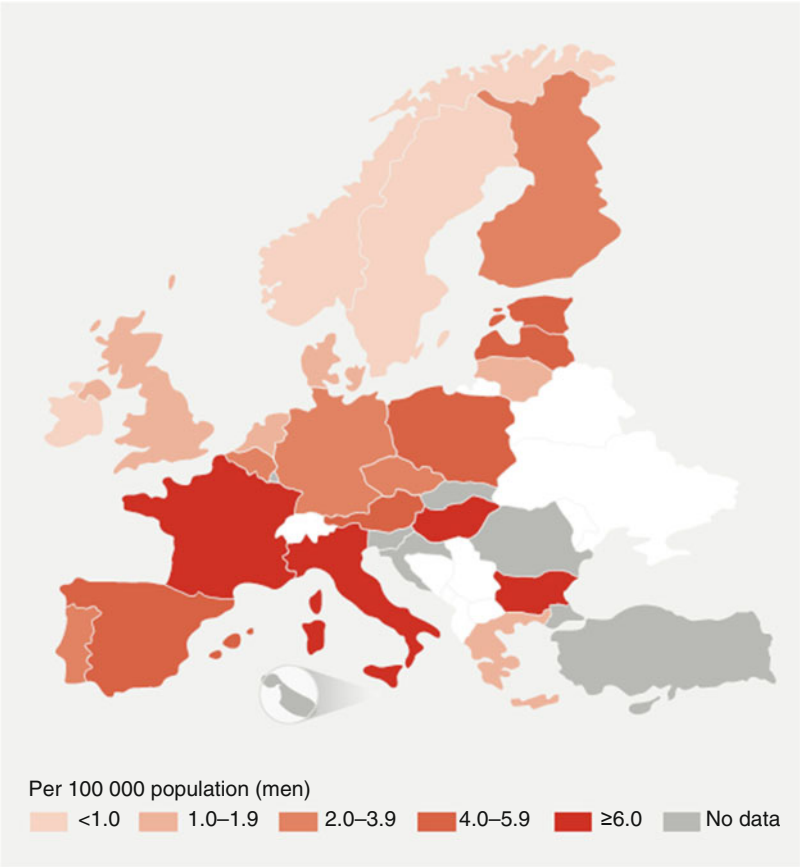


Fig. 6.6 Hepatocellular carcinoma-related mortality per 100,000 population (men). Source: [37]

6.2.7 Prevalence and Incidence of Injecting/Number of People Who Inject Drugs

Estimates of the numbers of PWID are important in projecting the future epidemiology of HCV infection and in planning and evaluating the public health responses [37]. A recent systematic review reported on the global prevalence of PWID [32], and as of June 2017, evidence of injecting drug use was reported in 179 of 206 countries or territories, an increase of 31 countries since a previous review of PWID prevalence [93]. The additional countries were mostly in sub-Saharan Africa ($n = 23$) and four Pacific Island states and territories. Globally, in 2015, an estimated 15.6 million people (95% uncertainty interval 10.2–23.7 million) injected drugs, amounting to approximately 0.33% (0.21–0.49) of those aged 15–64 years, and 21% of these were women (3.2 million, 1.6–5.1). At a regional level, prevalence varied

from 0.09% (0.07–0.11) in South Asia to 1.30% (0.71–2.15) in Eastern Europe. The largest populations of PWID were in East and Southeast Asia (4.0 million, 3.0–5.0 million), Eastern Europe (3.0 million, 1.7–5.0 million) and North America (2.6 million, 1.5–4.4 million). The proportion of women PWID varied substantially across regions—women were estimated to represent 30.0% (28.5–31.5) of PWID in North America and 33.4% (31.0–35.6) in Australasia, compared with 3.1% (2.1–4.1) among PWID in South Asia. The prevalence of injecting drug use among men was far higher than in women in all regions. The review found substantial variation in the estimated country-level prevalence of injecting drugs, with Georgia and Seychelles having the highest estimates; however, Russia, the USA and China contributed the largest proportions to the total number of PWID. Much lower prevalence was estimated for countries in Asia and sub-Saharan Africa than in other regions, though with some exceptions (Fig. 6.7) [32].

Relatively recent (2009–2015) national estimates of the prevalence of drug injecting among the general population are available in 16 of the 30 countries monitored by the EMCDDA [36]. Estimated prevalence varies across countries: from less than 1 to up to 9/1000 population aged 15–64 years (Fig. 6.8) although uncertainty intervals are often broad. Based on the available estimates, the highest absolute numbers of current PWID are reported in the UK (122,900), France (105,000), the Czech Republic (45,600), Finland (15,600), Portugal (14,400), Latvia (12,600) and Spain (9900). These numbers are important as they provide a proxy for the size of the group at potential risk of infection and transmission of HCV through injecting drug use. Combining estimates of injecting drug use with HCV prevalence estimates can enable us to understand the size and dynamic of the infection among this group [37].

6.2.8 Prevention and Harm Reduction for People Who Inject Drugs

Initiatives to reduce the spread of infectious diseases through the sharing of syringes and other drug injecting equipment by providing sterile drug use equipment to PWID date back to the mid-1980s [37, 94, 95]. This form of ‘prevention’ or ‘health protection’ activity is often in the policy context referred to as a ‘harm reduction’ approach, reflecting the fact that this response is not primarily focused on stopping the use of drugs but rather the prevention of harms associated with drug use. At present, most implemented interventions of this type are opiate substitution therapy (OST) and needle and syringe programmes (NSP). These two interventions when combined with high coverage have been associated with a reduction in the incidence of acute HCV infection [38, 96]. However, the coverage of these harm reduction responses, which are in place to some degree in a majority of the world’s countries, typically falls far short of what is needed to reach most PWID [97].

A recent systematic review reported on the global coverage of NSP and OST for PWID [98]. The study identified evidence of NSP operating in 93 of the 179 countries and territories where injecting drug use is known to occur (i.e. in 52% of countries where injecting drug use is reported). NSP was confirmed to be absent in 83 countries where injecting drug use occurs; the presence or absence of

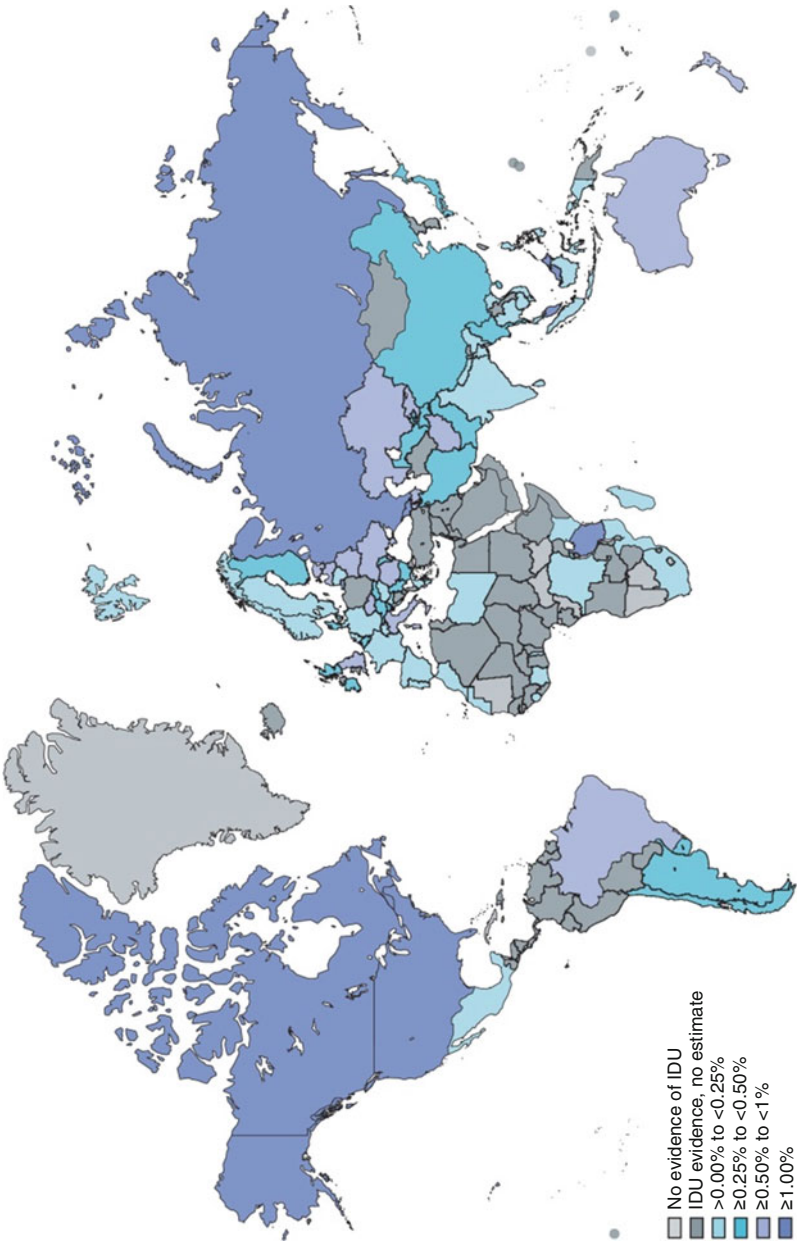


Fig. 6.7 Estimated prevalence of injecting drug use by country. Source: [32]. *IDU*: injecting drug use

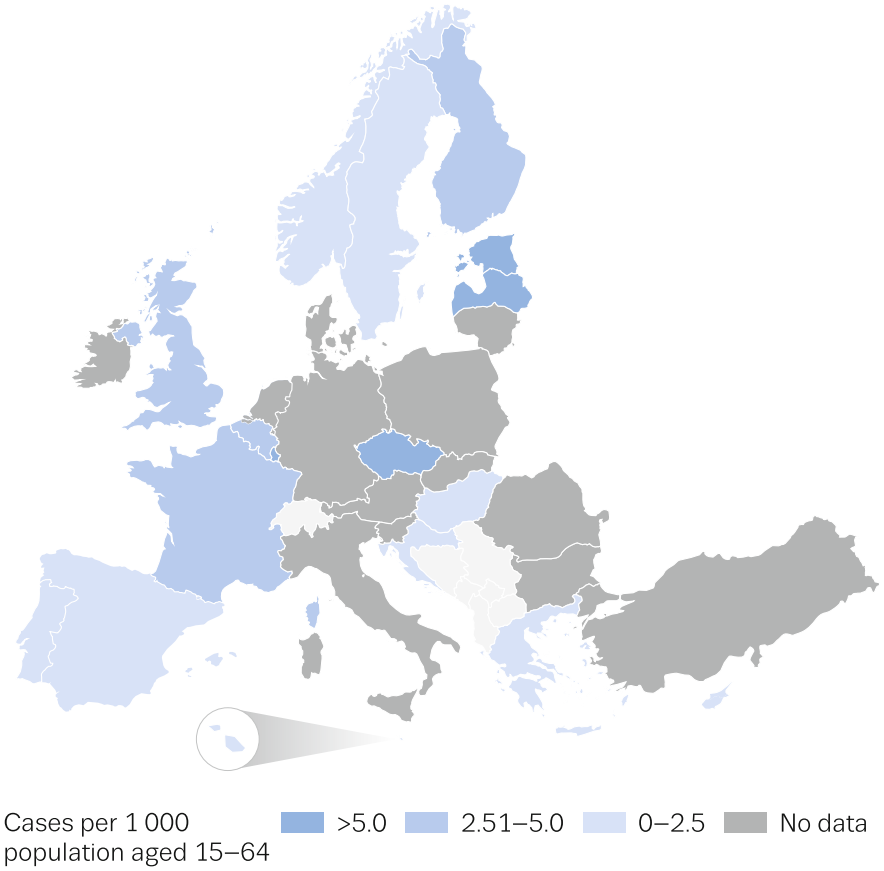


Fig. 6.8 Estimates of the prevalence of injecting drug use in Europe, 2009–2015 (most recent data). Source: [35]

NSP where injecting drug use is thought to occur could not be confirmed in three countries. OST was confirmed to be available in 86 countries where injecting drug use is known to occur (48% of countries where injecting drug use is reported), confirmed to be absent in 92 countries where injecting occurs, while the presence or absence of OST where injecting occurs could not be confirmed in one country. Methadone was the most frequently available medication used in OST, prescribed in 81 countries. Buprenorphine was prescribed for OST in 56 countries (of which 52 also prescribed methadone), and diamorphine was prescribed in seven countries (all of which also prescribed methadone and buprenorphine). Other forms of OST (e.g. tincture of opium, slow-release morphine) were prescribed in 12 countries. There were 79 countries implementing both NSP and OST (44% of countries where injecting drug use is reported) (Figs. 6.9 and 6.10) [98].

Fig. 6.9 Global coverage of needle and syringe programmes among people who inject drugs. Source: [32]. *NSP*: needle and syringe programmes, *PWID*: people who inject drugs

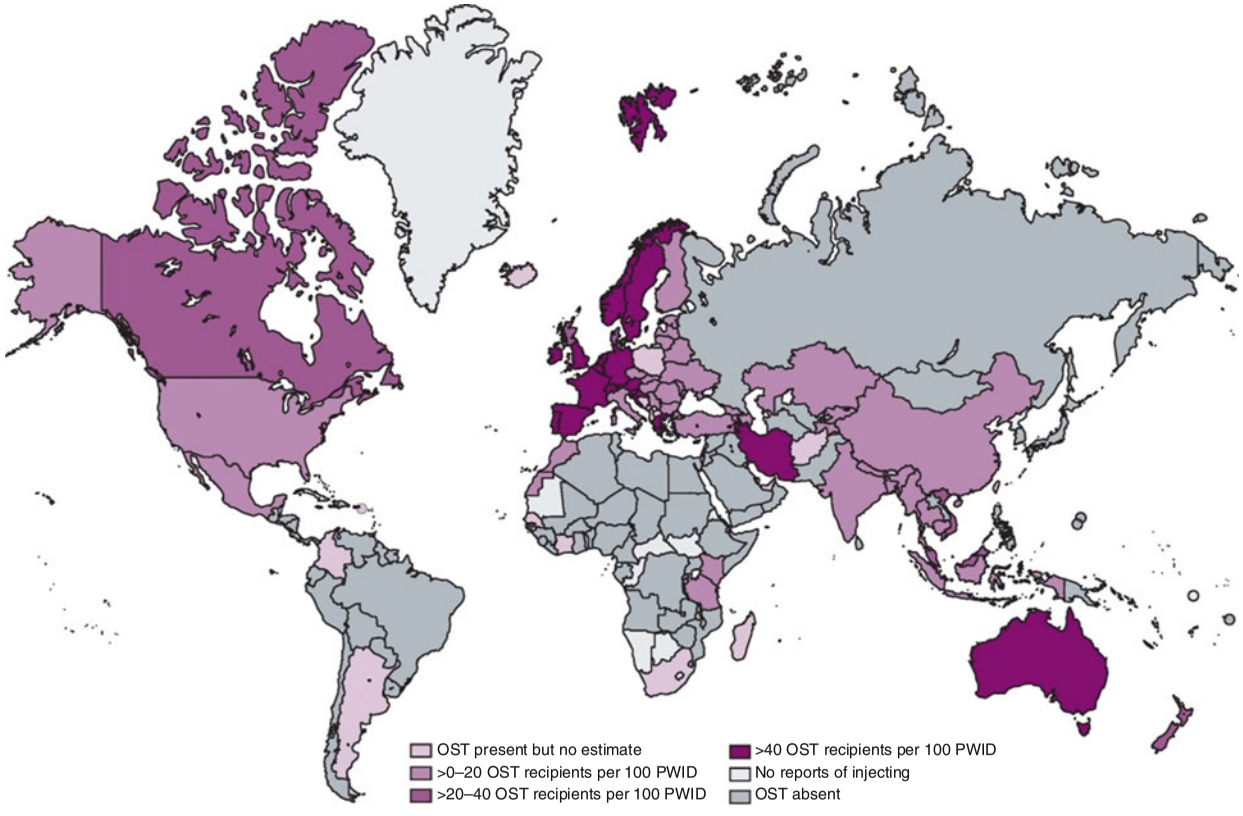


Fig. 6.10 Global coverage of opioid substitution therapy among people who inject drugs. Source: [32]. *OST*: opioid substitution therapy, *PWID*: people who inject drugs

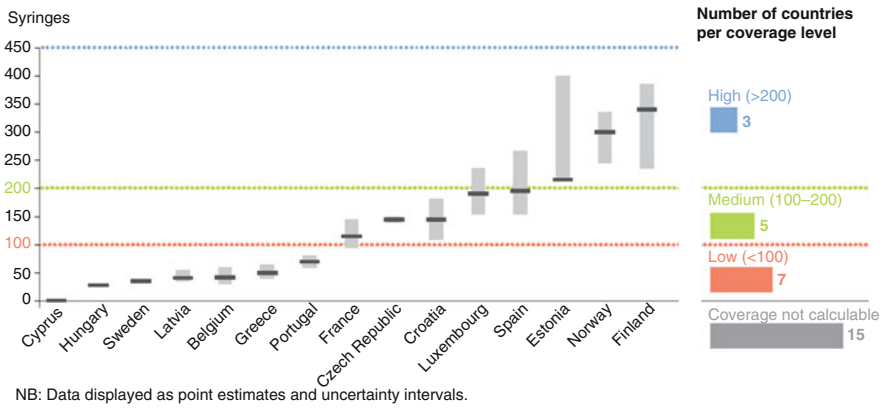
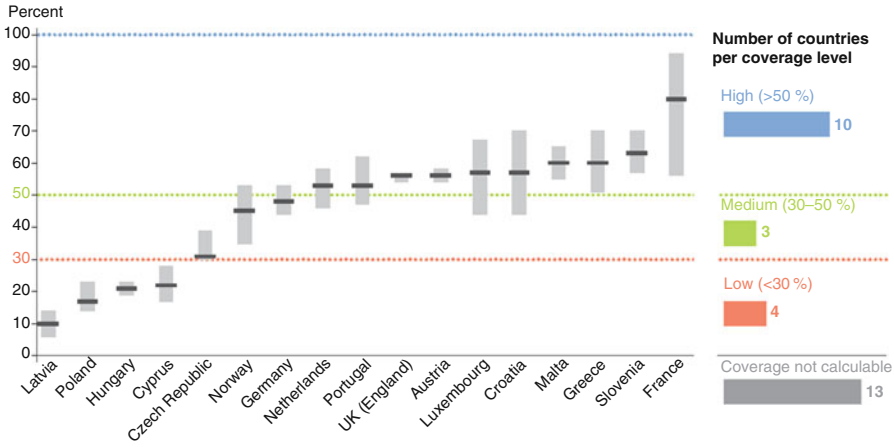


Fig. 6.11 Number of syringes provided through specialised needle and syringe programmes per estimated drug injector in 2015 or latest available year. Source: [36]

Coverage varied widely between countries, but was most often low according to WHO indicators (<100 needle-syringes distributed/PWID/year; <20 OST recipients/100 PWID/year). Globally, the study estimated that there are 33 (uncertainty interval 21–50) needle-syringes distributed via NSP per PWID annually and 16 (10–24) OST recipients/100 PWID. Less than 1% of PWID live in countries with high coverage of both NSP and OST (>200 needle-syringes distributed/PWID and >40 OST recipients/100 PWID) [98].

In Europe, needle and syringe programmes, integrated into multicomponent harm reduction interventions, distribute tens of millions of syringes each year. In addition to sterile syringes and needles, a range of other injecting paraphernalia, including alcohol pads, water, filters and mixing containers as well as equipment for inhaling drugs, are distributed by harm reduction facilities in order to prevent infections. The estimated number of syringes distributed each year per PWID through specialised programmes—excluding syringes sold by pharmacies outside of such programmes—ranged from less than 50 in Cyprus, Sweden, Belgium and Latvia to more than 350 in Estonia (Fig. 6.11). Comparing these estimates of syringe provision against international recommendations, less than a third of the countries that can be assessed provide syringes at a level judged to support effective harm reduction (at least 200 syringes/year/PWID [99]). Overall, it is estimated that approximately one in two high-risk opioid users in Europe received substitution treatment in 2014 [100]. This is the case for 10 of the 20 countries able to provide recent data allowing national coverage to be estimated. However, the available data indicate that in some countries, less than 10% of the estimated population of high-risk opioid users receive opioid substitution treatment (Fig. 6.12).

Exposure to disinfectants, including those containing alcohol, will effectively inactivate dried HCV on surfaces [75]. In a laboratory study simulating drug injection, containers (spoons or cookers) to prepare drugs for injection were contaminated with HCV in a water solution and heat was applied. The experiments



NB: Data displayed as point estimates and uncertainty intervals.

Fig. 6.12 Percentage of the estimated population of high-risk opioid users receiving substitution treatment in 2015. Source: [36]

showed that HCV could survive temperatures up to 65–70 °C, which required between 80 and 95 s of heating [75, 101].

In an earlier study in New York and Denver, ethnographers directly observed drug preparation in injection settings and measured heating times and temperatures applied to drug containers [102]. Only 12% of PWID heated drug solutions for >45 s, and nearly half heated for <15 s; they replicated these conditions in the laboratory and found that HIV was rapidly inactivated when heated, within 7–10 s. Thus, drug preparation practices that include heating may reduce the risk of HIV transmission via the shared use of containers, but HCV may still be transmitted [75].

This would also be consistent with substantial epidemiologic evidence that sharing containers is associated with HCV seroconversion [49, 77, 78]. However, it is not clear at present if this indicates direct transmission through contaminated injecting paraphernalia or if this association reflects syringe-mediated contamination when drugs are shared [79]. In a laboratory analysis of injection materials (syringes, drug cookers, filtration cotton, used water vials and alcohol and cotton swabs) collected from PWID in France, HCV RNA was not detected on used filters or water vials and was seldom detected on cups (9%). However, HCV RNA was frequently found on syringe pools (38%) and on swabs (82%) at high titres [75, 103]. Another laboratory study [104] demonstrated that HCV can survive for up to 3 weeks in bottled water and that HCV is also associated with filter material, in which around 10% of the viral inoculum was detectable. However, in a laboratory study attempting to replicate real-world injection practices, HCV could not be recovered from 'cookers', regardless of input syringe type or 'cooker' design. Recovery was higher when comparing detachable needles to fixed needles for residue in input syringes (73.8% vs. 0%), filters (15.4% vs. 1.4%) and receptive syringes (93.8% vs. 45.7%) [79].

A recent study [105] measured infectivity of laboratory clones of HCV recovered from used syringes and reported that 'HCV survival was dependent on syringe type, time and temperature'. Time and temperature may also have affected detection of HCV RNA in different types of equipment to a varying degree. Syringe type can determine the amount of blood remaining in a syringe when the plunger is fully inserted after injection, and this can range between 2 and 84 μl [75, 106].

Earlier studies of the use of disinfectant bleach to prevent HIV transmission via syringes shared by PWID suggested that, despite effectiveness claims in laboratory studies, this approach is ineffective in real-life settings [75, 107]. A recent study showed that HCV can be inactivated by microwave [108].

A project to assess tools to change the route of administration of drugs (e.g. heroin) from injecting to inhalation was conducted in Germany from 2011 to 2014 [109]. The aim of changing the route of administration was to reduce overdoses and the transmission of infections like HCV. A media campaign with posters, brochures, flyers and videos accompanied the project, and it was observed that PWID are willing to change their behaviour if well-equipped and sufficiently informed. Safer Smoke-Packs were developed, containing foil, straws and a flyer. Similar approaches have been piloted and are used in other countries [110].

A further intervention used to reduce drug use related harms, and often advocated for reducing drug overdose, is the provision of supervised injecting facilities. Such supervised injecting facilities are professionally run healthcare facilities where hygienic and safer use is promoted to reduce the morbidity and mortality associated with drug injecting [111, 112]. The facilities provide opportunities for health education and disease prevention and for immediate intervention by professionals in cases of overdose. Research has shown that supervised injecting facilities reach specific hard-to-reach target groups and that service users report substantial reductions in risk behaviour as well as improved health. Health promotion should include information which clarifies routes of transmission for diseases that are common among PWID. Information on infections like HIV, HCV and HBV should be provided, so that people understand that they can transmit the virus even if they show no symptoms [113].

6.2.9 HCV Treatment of People Who Inject Drugs

To date (2017), PWID in many countries have been excluded from HCV treatment. Despite multiple studies providing evidence that this population can be successfully treated [15, 114–121], HCV treatment rates among PWID are generally reported as low (<3%) even in high-income countries, due to concerns of poor adherence, psychiatric comorbidity and reinfection [122–125] and, especially since the introduction of DAAs, also costs. Prior to the introduction of DAAs, only 1–6% of HCV-infected current and former PWID in the USA, Canada and Australia were treated [3, 15, 28, 118, 119, 123, 126, 127]. International guidelines (such as the US National Institutes of Health, AASLD/IDSA, European Association for the Study of the Liver (EASL), International Network on Hepatitis in Substance Users and the

World Health Organization) all support treating HCV in people who use drugs [128–130]. Nevertheless, a recent study in the USA found that in 2014, 88% of states included drug and/or alcohol use in their HCV treatment eligibility criteria, with 50% requiring a period of abstinence and 64% requiring urine drug screening [28, 131].

A European review of treatment uptake before the widespread introduction of DAAs found that among groups of drug-using study participants who were HCV antibody-positive, the median treatment uptake level was 17%, and among those who were HCV RNA-positive, the median was 30%. In the 11 studies reporting specifically on treatment uptake among current and former PWID, HCV RNA-positive study populations had a median treatment uptake level of 32%. Only one study reported on HCV treatment uptake for people currently using drugs and found that uptake was relatively low among this group in several European countries and also pointed to considerable knowledge gaps regarding treatment uptake levels in this population [132]. A study from Germany however showed that direct-acting antiviral treatment of former or current drug users with or without opioid substitution therapy can achieve equally high sustained virologic response (SVR) rates as in patients with no history of drug use [133].

Since 2014, highly effective DAAs have been readily available. However, these treatments are costly and high costs could become a barrier to the widespread scale-up of HCV treatment. Guidelines issued by the European Association for the Study of the Liver (EASL) in 2015 recommend, for the first time, that treatment should be provided to PWID who are currently injecting on account of their risk of transmitting infection to others ('treatment as prevention'), irrespective of disease stage [134].

Several theoretical modelling studies have explored the potential impact and benefits of HCV treatment as prevention among PWID populations [28]. Modelling projections have indicated that achieving substantial reductions in HCV prevalence among PWID requires HCV treatment in addition to primary prevention. In addition to individual benefits, model projections have shown that HCV treatment for PWID could be an effective and cost-effective means of prevention in settings where chronic HCV prevalence among this group is less than 60% and that PWID should be prioritised after treating people with severe liver disease [135–141].

Interestingly, there is insufficient evidence to date of any impact of HIV treatment as prevention among marginal at-risk populations such as PWID [142, 143]. However, in theory, HCV treatment as prevention could be more effective than HIV treatment as prevention because HCV treatment is finite and curative. In particular, the dramatic improvement in SVR rates, once-daily dosing and short therapies (8–12 weeks) with interferon-free direct-acting antiviral therapies (IFN-free DAAs) has led many to speculate whether HCV treatment could feasibly be scaled up sufficiently to be used as an effective prevention strategy among those at risk of transmission [28, 144–148].

In most countries, it will be essential to scale up HCV treatment if the increasing trend in the prevalence of end-stage liver disease is to be reversed [45, 149]. However, targeting people with cirrhosis, as is the priority in many European countries, is unlikely to lead to substantial reductions in HCV transmission or the prevalence of

HCV infection among PWID [150, 151] as by the time cirrhosis has developed, injecting drug use behaviour has usually ceased. Although much of the HCV treatment as prevention modelling work has been done in a few countries (Australia, Canada, France, UK), the scenarios reflect the situation in many European cities and, therefore, can be generalised [141].

6.3 Men Who Have Sex with Men

Since 2000, outbreaks of acute HCV among HIV-positive MSM who have not reported injecting drug use have been published from Europe [152–159], the USA [160–162] and Australia [163]. The majority of these HCV infections were related to permucosal rather than parenteral risk factors, providing further evidence to support the role of sexual transmission [17]. These outbreaks have been associated with high-risk sexual practices, genital ulcer disease and illicit drug use including parenteral administration [17, 158, 164, 165]. However, a recent study in Amsterdam suggests that HIV-negative MSM may also be at risk of HCV infection, with the same HCV strains already circulating among HIV-positive MSM [166].

In 17 studies (from Australia, Canada, China, Denmark, Italy, Japan, the Netherlands, Spain, Switzerland, Taiwan, the UK and the USA), more than 13,000 HIV-positive MSM were followed for over 91,000 PY between 1984 and 2012; the pooled seroconversion rate was 0.53/100 PY. Calendar time was a significant moderator of HCV seroconversion, increasing from an estimated rate of 0.42/100 PY in 1991 to 1.09/100 PY in 2010 and 1.34/100 PY in 2012. Among those who seroconverted, a large proportion of infections were attributable to high-risk behaviours including mucosa-traumatic sex and sex while high on methamphetamine [167].

In Europe, during the past decade, the incidence of HCV in cohorts of HIV-positive MSM rose from 0.08/100 PY of follow-up (PYFU) to 4.1/100 PYFU [157, 168–171]. Currently, the epidemic appears to be declining in the Netherlands where a study in HIV treatment centres showed a decrease in incidence from 1.1 to 0.5/100 PFYU between 2014 and 2016 [172, 173]. However, in France, a study in a large cohort of people living with HIV found that despite a high HCV treatment uptake and cure rate, the incidence of new HCV infection (first infection or reinfection) regularly increased in French HIV-positive MSM between 2012 and 2016. First infection incidence in MSM rose from 0.5% to 0.92% patient-years, whereas the incidence of reinfection fluctuated but remained higher than the incidence of first infection (2.52–2.90% patient-years), suggesting that a subgroup of MSM pursued high-risk practices following cure of a first infection [174]. Outside Europe (the USA and Japan), no levelling off seems to be observed either, with recent reported incidence rates being between 0.2/100 PYFU and 2.5/100 PFYU [175, 176]. The acute HCV reinfection rate is even higher, with reported rates of 7.8 and 15.2/100 PYFU [31, 177, 178].

A supposed reason for this increase is the emergence of national and international networks of HIV-positive men who have unprotected sex with other HIV-positive

men ('serosorting') [179]. These could have arisen because of successful HIV treatment, widespread Internet use and low-budget travel possibilities [157, 180]. More recently, with effective ART, PreP and PEP serosorting may have declined, which could explain the emergence of HCV among HIV-negative MSM. Reported determinants for HCV transmission are sexualised drug use (including 'chemsex'); sharing of snorting straws; receptive fisting; ulcerative sexually transmitted infections, such as syphilis; group sex; and rectal trauma with bleeding [158, 164, 170, 181]. There are also a number of potential mechanisms related to HIV that might result in enhanced infectivity of and susceptibility to HCV, including increased HCV loads in serum and semen, and defects in the gastrointestinal immune system [17, 31].

6.4 Patients at Risk of Nosocomial Infection

Before the identification of HCV, transfusion of blood or blood-related products was one of the main routes of its transmission. Routine anti-HCV antibody screening in blood donations has almost eliminated the risk of HCV transmission from blood donations in many countries worldwide. However, there remains variation in routine testing for transfusion-related infections worldwide, and screening of blood donations is not conducted at all in around 40 countries due to financial restrictions with inconsistent testing in many other countries [182].

Unsafe injection, principally due to equipment reuse, in healthcare settings is also a risk for HCV. The WHO estimates that around two million new infections each year result from unsafe injections, accounting for 40% of all new infections [55, 183]. In 2015, it was estimated that, globally, 5% of healthcare-related injections remained unsafe [1].

The most dramatic example is Egypt, in which the iatrogenic transmission of HCV during the era of parenteral antischistosomal therapy mass treatment between 1960 and 1980 led to a nationwide epidemic. As a result, 10–20% of the total Egyptian population are currently infected, most of them with genotype 4. Because of inadequate sterilisation of healthcare equipment (and presumably other breaches in infection prevention and control in healthcare) and the high prevalence in the general population, HCV continues to spread in Egypt [29, 31].

Within the European healthcare systems, HCV transmission still occurs. For example, according to the national surveillance system in France, these infections (mainly from invasive procedures) account for up to 25% of all acute HCV infections diagnosed [184]. In this study, suspected healthcare procedures were mainly surgery, haemodialysis and endoscopy, a finding consistent with previous studies in France and Italy [184–188]. However, for hospitalised patients in Europe, the risk of acute HCV infection via blood transfusion or via medicinal use of contaminated needle injections has declined to low levels [31].

In most countries with available data, HCV prevalence is higher in men than women; this finding reflects the higher prevalence of risk factors (such as injecting drug use) in men than in women. In France, however, more women are

infected than men [189]. Similarly, in Germany, more women aged over 69 years have HCV infection than age-matched men [189]. In both countries, women were at risk of infection during childbirth in the late 1970s via contaminated blood or equipment. HCV is more common in women than men in Turkey; most infections in Turkey are nosocomial, with hospitalisation more common in women than in men [55, 189].

6.5 Migrants

Migrants born in countries with an intermediate or high HCV prevalence are at risk of having a chronic infection, mainly due to a higher risk of nosocomial transmission in the country of origin. A systematic review on the HCV prevalence among migrants worldwide showed the anti-HCV prevalence was high (>3%) in migrants from South Asia and sub-Saharan Africa, and intermediate (2–3%) in migrants from Eastern Europe and Central Asia [26].

The numbers of chronically infected migrants in the EU/EEA and the UK by country of birth and the contribution of migrants to the overall burden of disease have been estimated for the year 2013 [190]. In 2013, around 11% of the total population in the EU/EEA was foreign born to their country, of which 79% was born in HCV-endemic countries (anti-HCV prevalence ≥1%). The anti-HCV prevalence among migrants in the EU/EEA and the UK from HCV endemic countries was 2.3%, corresponding to around 580,000 chronic HCV infections. While 1 in 12 people in the EU/EEA and the UK is born in an HCV endemic country, migrants from endemic countries account for 1 in 7 (14%) of the total number of HCV infections in the EU/EEA and the UK. The relative contribution of migrants is higher in countries with a low HCV prevalence in the general population and with high numbers of migrants from countries of higher prevalence; in, for example, Germany and the Netherlands the proportion of all HCV infections that are among migrants is estimated to exceed 50% of the total number of chronic HCV infections [190].

6.6 Reinfection

The prospects of eliminating HCV could be counteracted by HCV reinfection in those successfully treated, and those who have naturally cleared infection, due to continued risks [71, 191]. This has been described in PWID and MSM [4, 165, 177, 192, 193].

Documentation of high rates of reinfection after treatment among HIV-HCV-co-infected MSM (8–15/100 PY) [165, 177, 178, 194] as well as evidence of a highly connected global network of HCV transmission due to travel may limit the effectiveness of treatment as prevention strategies in this key risk group [28]. Reinfection post-successful HCV treatment ($n = 2$ studies) among MSM was 20 times higher than initial seroconversion rates [167]. A study from eight HIV treatment centres in four European countries found a trend for lower incidence among MSM who had

spontaneously cleared their incident infection (5/100 PYFU) than among those who were treated (8/100 PYFU) [165].

Based on existing data from small and heterogeneous studies of interferon-based treatment, the incidence of reinfection after sustained virological response ranged from 2–6/100 PY among PWID to 10–15/100 PY among MSM with HIV [71].

In a recent meta-analysis of 61 studies published in 1990–2015, the 5-year risk of HCV reinfection in HIV-infected MSM was as high as 15% and higher than in studies on PWID [165, 195]. However, another recent study found that when accounting for frequency of risk behaviour, those reporting high-frequency injecting drug use had the highest risk (adjusted reinfection rate (per 1000 PYFU): 58, 95% credible interval [CrI], 18–134), followed by MSM reporting high-risk sexual activity (26, 95% CrI, 6–66) and low-frequency injecting drug use [196].

More recently, HCV reinfections have also been reported in phase III trials of DAA HCV compounds [197–199], nearly all of which have occurred among HIV-infected MSM [165].

6.7 Discussion

Globally, HCV incidence is mainly driven by two different mechanisms: in many developing countries, the use of unsafe invasive medical practice and lack of testing blood donations are key, while in many high- and middle-income countries, specific behaviours in high-risk groups such as PWID and MSM predominate. This calls for diversified prevention and monitoring strategies: in the first case, general population-based epidemiology and treatment with prevention focused on healthcare settings, while in the second case risk, group-based epidemiology, prevention and treatment are indicated.

Large-scale investment in awareness campaigns and education of the general public and healthcare staff about the risks associated with reuse of medical instruments might be one way to make inroads into this widespread ongoing problem. Another critical step in the control of the global burden of HCV is identifying and testing at-risk persons for HCV in each country. However, this task is daunting: it is estimated that 90% of the HCV-infected individuals worldwide are unaware of their infection status [55, 200, 201]. In 2017, the first WHO guidelines on hepatitis B and C testing were published recommending focused testing of individuals with (a history of) high-risk behaviour or who are part of a population with a higher seroprevalence [202]. A general population testing approach is recommended in settings with an anti-HCV prevalence of $\geq$2% or 'birth cohort' testing for specific age groups with a higher prevalence. A range of operational interventions that can enhance testing, linkage to care and treatment and thereby substantially optimise the continuum of care for chronic viral hepatitis were identified in a recent systematic review of studies, (all of which except one) from high-income countries. Findings included the following: clinician reminders to prompt HCV testing during clinical visits increased HCV testing rates; nurse-led

educational interventions improved HCV treatment completion and cure; and coordinated mental health, substance misuse and hepatitis treatment services increased HCV treatment uptake, adherence and cure compared with usual care [203].

The immediate priority is to scale up HCV treatment in people with severe liver disease to reduce HCV-related morbidity and mortality, as rapidly as possible. Thereafter, the question is which patients should be prioritised next for treatment—should countries target those with moderate liver disease (pre-cirrhotic) or those with HCV who are currently injecting drugs (or HIV-positive MSM), most of whom will have no or mild disease, as recommended by EASL. A recent EMCDDA publication emphasises the importance of HCV treatment in PWID—it is unlikely that the combination of opioid substitution treatment and needle and syringe programmes in itself will achieve substantial reductions in HCV prevalence in this group [141]. So far, economic modelling supports treatment for and prioritisation of PWID, as essential for achieving elimination targets. The evidence suggests that prioritising early HCV treatment on PWID can be highly cost-effective, depending on the prevalence of HCV [139]—but as yet we lack direct empirical evidence (i.e. that HCV transmission is reduced as a result of scaling up HCV treatment) [134].

While improvements in screening and treatment are becoming a priority in new HCV strategies in some countries, there is evidence that HCV is not being addressed in a comprehensive manner, as several countries still show important gaps in prevention coverage, and HCV treatment provision to PWID continues to be reported as low [37]. In high- and middle-income countries, it is clear that the highest proportion of infected individuals are former or current PWID and that treatment of infection is needed in this group. Substantial reductions in HCV incidence and prevalence can only be achieved with targeted DAA therapy among those at the highest risk of ongoing transmission [204]. However, despite the availability of novel treatment options with improved efficacy and tolerability, treatment is limited in this group. A recent study of the readiness in European countries to treat hepatitis C virus in individuals with opioid use disorder, on the basis of an expert-generated model assessment, showed that there are important limitations to successful HCV care in people with opioid use disorders, which most PWID are. According to the experts, specific actions should be taken: maintain/increase access to opioid use disorders treatment services/opioid agonist therapy, update HCV guidance, locate care in the same place and allow wider prescribing of anti-HCV medicines [205].

Regarding PWID and HIV-infected MSM, mathematical modelling predicts that if the required scale-up in treatment uptake with the new treatments is achieved, the result would be substantial reductions in HCV prevalence within a decade [138, 206]. Further benefits have been predicted if treatment is combined with an intervention to reduce behavioural risk, which makes the eradication of HCV an achievable goal in the HIV–HCV-coinfected population in Western Europe [165]. Among MSM, there is a lack of evidence-based behavioural interventions to reduce risk behaviours which have been associated with HCV transmission (such

as sexual and drug practices associated with mucosal trauma). Therefore, additional prevention interventions in these populations are urgently needed [28].

In contrast to PWID, the absolute numbers of HCV–HIV-coinfected MSM are small, and most diagnosed HIV-positive MSM are linked with care, closely monitored and frequently tested. Additionally, high uptake of HCV treatment among HIV-positive MSM has been reported, with over 40% of HIV–HCV-coinfected MSM being treatment experienced in European cohorts [153, 207, 208]. Hence, HCV treatment for prevention may be particularly feasible in this group [28].

However, according to WHO, other key populations need to be included in national strategies and need to be actively screened, diagnosed and linked to treatment and prevention of reinfection, e.g. migrant communities originating from countries with intermediate or high HCV prevalence [202].

Vaccinating key population groups at risk for HCV infection, or already infected, against other hepatitis viruses, in particular hepatitis B, should be considered by policy makers in accordance with local guidelines. Because of the possibility of a higher risk of hepatitis A outbreaks among PWID, the provision of a combined hepatitis A and B vaccination is suggested as the best way to prevent both infections in PWID and to avoid additional harm of the liver. This is particularly important for those who are HCV-positive.

Evidence indicates that the prevalence of HCV-related end-stage liver disease and mortality is increasing. However, the prevalence of severe liver disease among PWID with HCV remains largely unknown. Nor is it clear how many PWID have been treated for HCV infection. In addition, knowledge of the coverage of other key HCV primary interventions—opioid substitution treatment and needle and syringe programmes—is patchy in many countries, and in many European countries there are no reliable estimates of the population currently at risk of HCV infection through injecting drug use. Developing better surveillance and evidence on HCV is important and will require collaboration between international and regional organisations and among individual countries [134].

With the new treatments, national and international strategies are required to redesign and co-locate treatment services for managing HCV infection with specialist drug services for PWID. However, this is only the first step in addressing stigma and promoting patient-facing treatment services for PWID [209]. Certainly, there is now a window of opportunity to generate empirical data and conduct evaluations of the impact of scaling up HCV treatment among PWID in European settings, as treatment services are geared up to identify and deal with severe liver disease. Ideally, potential intervention sites will have established ‘HCV treatment-in-the-community’ services, integrated with other services that manage and support PWID, and critically sites will need to have mature systems for collecting data on behaviour, HCV transmission and HCV prevalence among this client group, and on HCV testing and treatment. In the context of the EASL guidelines and the changing therapeutic landscape of HCV, such an evaluation needs to be done as quickly as possible [134].

The burden of HCV is high and disproportionately affects PWID. In many countries with available data, more than half of PWID are infected, and current

data indicate ongoing transmission. The European picture is highly variable, with large variations in both the epidemiology of the infection and the prevention responses undertaken [3]. The coverage of interventions in some countries continues to be low when measured against international standards, and, in some instances, it has even been recently decreasing, significantly increasing the risk of HCV and other infections among PWID. There are significant gaps and also general limitations in the available data on notifications, prevalence estimates, estimates of the numbers of people injecting drugs and coverage of the main prevention interventions. Serious gaps also exist in estimates of incidence, co-infection, genotypes, undiagnosed fraction, treatment entry and burden of disease [3]. All these are valuable indicators for monitoring the continuum of care, and they should be promoted and their availability improved in several countries where they are still underdeveloped [3, 35, 37].

There is a need now to generate empirical data and conduct evaluations of the impact and cost-effectiveness of scaling up HCV treatment among people who inject drugs in European settings [141]. Constructive prevention strategies include acknowledgement of the problem without stigma and discrimination as a crucial first step, education and counselling, harm reduction optimisation, scaled-up treatment including treatment of injecting networks, post-treatment screening and rapid retreatment of reinfections [71].

References

1. WHO. Global hepatitis report, 2017. Geneva: World Health Organisation; 2017. http://apps.who.int/iris/bitstream/10665/255016/1/9789241565455-eng.pdf?ua=1.
2. Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis. 2005;5(9):558–67.
3. Wiessing L, Ferri M, Grady B, Kantzanou M, Sperle I, Cullen KJ, et al. Hepatitis C virus infection epidemiology among people who inject drugs in Europe: a systematic review of data for scaling up treatment and prevention. PLoS One. 2014;9(7):e103345. https://doi.org/10.1371/journal.pone.0103345.
4. Grebely J, Prins M, Hellard M, Cox AL, Osburn WO, Lauer G, et al. Hepatitis C virus clearance, reinfection, and persistence, with insights from studies of injecting drug users: towards a vaccine. Lancet Infect Dis. 2012;12(5):408–14.
5. Burke KP, Cox AL. Hepatitis C virus evasion of adaptive immune responses: a model for viral persistence. Immunol Res. 2010;47(1–3):216–27.
6. Micallef JM, Kaldor JM, Dore GJ. Spontaneous viral clearance following acute hepatitis C infection: a systematic review of longitudinal studies. J Viral Hepat. 2006;13(1):34–41.
7. Rein DB, Wittenborn JS, Weinbaum CM, Sabin M, Smith BD, Lesesne SB. Forecasting the morbidity and mortality associated with prevalent cases of pre-cirrhotic chronic hepatitis C in the United States. Dig Liver Dis. 2011;43(1):66–72.
8. Perz JF, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. J Hepatol. 2006;45(4):529–38.
9. Hope VD, Eramova I, Capurro D, Donoghoe MC. Prevalence and estimation of hepatitis B and C infections in the WHO European Region: a review of data focusing on the countries outside the European Union and the European Free Trade Association. Epidemiol Infect. 2014;142(2):270–86.

10. WHO. Combating Hepatitis B and C to reach elimination by 2030. Geneva: WHO; 2016. http://apps.who.int/iris/bitstream/10665/206453/1/WHO_HIV_2016.04_eng.pdf.
11. WHO. Hepatitis C—Fact sheet No. 164. Geneva: World Health Organisation; 2017. http://www.who.int/mediacentre/factsheets/fs164/en/.
12. Falade-Nwulia O, Suarez-Cuervo C, Nelson DR, Fried MW, Segal JB, Sulkowski MS. Oral direct-acting agent therapy for hepatitis C virus infection: a systematic review. Ann Intern Med. 2017;166(9):637–48.
13. Pawlotsky JM. Treatment of chronic hepatitis C: current and future. Curr Top Microbiol Immunol. 2013;369:321–42.
14. Adeyemi OM, Jensen D, Attar B, Ghaoui R, Gallagher M, Wolen D, et al. Hepatitis C treatment eligibility in an urban population with and without HIV coinfection. AIDS Patient Care STDs. 2004;18(4):239–45.
15. Grebely J, Raffa JD, Lai C, Krajden M, Kerr T, Fischer B, et al. Low uptake of treatment for hepatitis C virus infection in a large community-based study of inner city residents. J Viral Hepat. 2009;16(5):352–8.
16. Memon MI, Memon MA. Hepatitis C: an epidemiological review. J Viral Hepat. 2002;9(2):84–100.
17. van de Laar TJ, Matthews GV, Prins M, Danta M. Acute hepatitis C in HIV-infected men who have sex with men: an emerging sexually transmitted infection. AIDS. 2010;24(12):1799–812.
18. Aceijas C, Rhodes T. Global estimates of prevalence of HCV infection among injecting drug users. Int J Drug Policy. 2007;18(5):352–8.
19. Nelson PK, Mathers BM, Cowie B, Hagan H, Des Jarlais D, Horyniak D, et al. Global epidemiology of hepatitis B and hepatitis C in people who inject drugs: results of systematic reviews. Lancet. 2011;378(9791):571–83.
20. Hajarizadeh B, Grebely J, Dore GJ. Epidemiology and natural history of HCV infection. Nat Rev Gastroenterol Hepatol. 2013;10(9):553–62.
21. Stephenson J. Former addicts face barriers to treatment for HCV. JAMA. 2001;285(8):1003–5.
22. Wiessing L, Guarita B, Giraudon I, Brummer-Korvenkontio H, Salminen M, Cowan SA. European monitoring of notifications of hepatitis C virus infection in the general population and among injecting drug users (IDUs)—the need to improve quality and comparability. Euro Surveill. 2008;13(21):pii: 18884. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18884.
23. Madhava V, Burgess C, Drucker E. Epidemiology of chronic hepatitis C virus infection in sub-Saharan Africa. Lancet Infect Dis. 2002;2(5):293–302.
24. Dolan K, Teutsch S, Scheuer N, Levy M, Rawlinson W, Kaldor J, et al. Incidence and risk for acute hepatitis C infection during imprisonment in Australia. Eur J Epidemiol. 2010;25(2):143–8.
25. Larney S, Kopinski H, Beckwith CG, Zaller ND, Jarlais DD, Hagan H, et al. Incidence and prevalence of hepatitis C in prisons and other closed settings: results of a systematic review and meta-analysis. Hepatology. 2013;58(4):1215–24.
26. Greenaway C, Thu MA, Kloda LA, Klein M, Cnossen S, Schwarzer G, et al. The seroprevalence of hepatitis C antibodies in immigrants and refugees from intermediate and high endemic countries: a systematic review and meta-analysis. PLoS One. 2015;10(11):e0141715.
27. Bradshaw D, Matthews G, Danta M. Sexually transmitted hepatitis C infection: the new epidemic in MSM? Curr Opin Infect Dis. 2013;26(1):66–72.
28. Martin NK, Vickerman P, Dore GJ, Hickman M. The hepatitis C virus epidemics in key populations (including people who inject drugs, prisoners and MSM): the use of direct-acting antivirals as treatment for prevention. Curr Opin HIV AIDS. 2015;10(5):374–80.
29. Paez JA, Sharaf EN, Rimlinger F, El-Daly M, El-Hariri H, El-Hoseiny M, et al. HCV iatrogenic and intrafamilial transmission in Greater Cairo, Egypt. Gut. 2010;59(11):1554–60.

30. Bunchorntavakul C, Jones LM, Kikuchi M, Valiga ME, Kaplan DE, Nunes FA, et al. Distinct features in natural history and outcomes of acute hepatitis C. J Clin Gastroenterol. 2015;49(4): e31–40.
31. Hullegie SJ, Arends JE, Rijnders BJ, Irving WL, Salmon D, Prins M, et al. Current knowledge and future perspectives on acute hepatitis C infection. Clin Microbiol Infect. 2015;21(8):797.
32. Degenhardt L, Peacock A, Colledge S, Leung J, Grebely J, Vickerman P, et al. Global prevalence of injecting drug use and sociodemographic characteristics and prevalence of HIV, HBV, and HCV in people who inject drugs: a multistage systematic review. Lancet Glob Health. 2017;5(12):e1192–207.
33. Micallef JM, Macdonald V, Jauncey M, Amin J, Rawlinson W, Van BI, et al. High incidence of hepatitis C virus reinfection within a cohort of injecting drug users. J Viral Hepat. 2007;14 (6):413–8.
34. Hahne SJ, Veldhuijzen IK, Wiessing L, Lim TA, Salminen M, Laar M. Infection with hepatitis B and C virus in Europe: a systematic review of prevalence and cost-effectiveness of screening. BMC Infect Dis. 2013;13:181. http://www.biomedcentral.com/1471-2334/13/181.
35. Hope V, Giraudon I, Kalamara E, Hedrich D. Drug-related infectious diseases in Europe. Update from the EMCDDA expert network. Lisbon: EMCDDA; 2017. https://goo.gl/rdSaBz.
36. EMCDDA. European Drug Report 2017. Lisbon: EMCDDA; 2017. http://www.emcdda.europa.eu/system/files/publications/4541/TDAT17001ENN.pdf_en.
37. Giraudon I, Hedrich D, Duffell E, Kalamara E, Wiessing L. Hepatitis C virus infection among people who inject drugs: epidemiology and coverage of prevention measures in Europe. Hepatitis C among drug users in Europe. Lisbon: EMCDDA; 2016. http://www.emcdda.europa.eu/system/files/publications/2953/TDXD16002ENN_final_web.pdf.
38. Turner KM, Hutchinson S, Vickerman P, Hope V, Craine N, Palmateer N, et al. The impact of needle and syringe provision and opiate substitution therapy on the incidence of hepatitis C virus in injecting drug users: pooling of UK evidence. Addiction. 2011;106(11):1978–88.
39. Hope VD, Hickman M, Ngui SL, Jones S, Telfer M, Bizzarri M, et al. Measuring the incidence, prevalence and genetic relatedness of hepatitis C infections among a community recruited sample of injecting drug users, using dried blood spots. J Viral Hepat. 2011;18 (4):262–70.
40. Balogun MA, Murphy N, Nunn S, Grant A, Andrews NJ, Teo CG, et al. Prevalence and incidence of hepatitis C in injecting drug users attending genitourinary medicine clinics. Epidemiol Infect. 2009;137(7):980–7.
41. Aarons E, Grant P, Soldan K, Luton P, Tang J, Tedder R. Failure to diagnose recent hepatitis C virus infections in London injecting drug users. J Med Virol. 2004;73(4):548–53.
42. Brant LJ, Ramsay ME, Balogun MA, Boxall E, Hale A, Hurrelle M, et al. Diagnosis of acute hepatitis C virus infection and estimated incidence in low- and high-risk English populations. J Viral Hepat. 2008;15(12):871–7.
43. Health Protection Agency, Health Protection Scotland, Public Health Wales, and Public Health Agency Northern Ireland. Health Hepatitis C in the UK, 2012 Report. London: Health Protection Agency; 2012.
44. Cullen KJ, Hope VD, Croxford S, Shute J, Ncube F, Parry JV. Factors associated with recently acquired hepatitis C virus infection in people who inject drugs in England, Wales and Northern Ireland: new findings from an unlinked anonymous monitoring survey. Epidemiol Infect. 2015;143(7):1398–407.
45. Razavi H, Waked I, Sarrazin C, Myers RP, Idilman R, Calinas F, et al. The present and future disease burden of hepatitis C virus (HCV) infection with today's treatment paradigm. J Viral Hepat. 2014;21(Suppl 1):34–59.
46. Hatzakis A, Chulanov V, Gadano AC, Bergin C, Ben-Ari Z, Mossong J, et al. The present and future disease burden of hepatitis C virus (HCV) infections with today's treatment paradigm—volume 2. J Viral Hepat. 2015;22(Suppl 1):26–45.

47. Sibley A, Han KH, Abourached A, Lesmana LA, Makara M, Jafri W, et al. The present and future disease burden of hepatitis C virus infections with today's treatment paradigm—volume 3. J Viral Hepat. 2015;22(Suppl 4):21–41.
48. Zibbell JE, Asher AK, Patel RC, Kupronis B, Iqbal K, Ward JW, et al. Increases in acute Hepatitis C virus infection related to a growing opioid epidemic and associated injection drug use, United States, 2004 to 2014. Am J Public Health. 2018;108(2):175–81.
49. Hagan H, Pouget ER, Williams IT, Garfein RL, Strathdee SA, Hudson SM, et al. Attribution of hepatitis C virus seroconversion risk in young injection drug users in 5 US cities. J Infect Dis. 2010;201(3):378–85.
50. Smith DB, Bukh J, Kuiken C, Muerhoff AS, Rice CM, Stapleton JT, et al. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource. Hepatology. 2014;59(1):318–27.
51. Gower E, Estes C, Blach S, Razavi-Shearer K, Razavi H. Global epidemiology and genotype distribution of the hepatitis C virus infection. J Hepatol. 2014;61(1 Suppl):S45–57.
52. Simmonds P. Genetic diversity and evolution of hepatitis C virus—15 years on. J Gen Virol. 2004;85(Pt 11):3173–88.
53. Fathi H, Clark A, Hill NR, Dusheiko G. Effectiveness of current and future regimens for treating genotype 3 hepatitis C virus infection: a large-scale systematic review. BMC Infect Dis. 2017;17(1):722.
54. Kanwal F, Kramer JR, Ilyas J, Duan Z, El-Serag HB. HCV genotype 3 is associated with an increased risk of cirrhosis and hepatocellular cancer in a national sample of U.S. Veterans with HCV. Hepatology. 2014;60(1):98–105.
55. Thrift AP, El-Serag HB, Kanwal F. Global epidemiology and burden of HCV infection and HCV-related disease. Nat Rev Gastroenterol Hepatol. 2017;14(2):122–32.
56. Petruzziello A, Marigliano S, Loquercio G, Cacciapuoti C. Hepatitis C virus (HCV) genotypes distribution: an epidemiological up-date in Europe. Infect Agent Cancer. 2016;11:53.
57. Kamal SM, Nasser IA. Hepatitis C genotype 4: what we know and what we don't yet know. Hepatology. 2008;47(4):1371–83.
58. Platt L, Easterbrook P, Gower E, McDonald B, Sabin K, McGowan C, et al. Prevalence and burden of HCV co-infection in people living with HIV: a global systematic review and meta-analysis. Lancet Infect Dis. 2016;16(7):797–808.
59. Barth RE, Huijgen Q, Taljaard J, Hoepelman AI. Hepatitis B/C and HIV in sub-Saharan Africa: an association between highly prevalent infectious diseases. A systematic review and meta-analysis. Int J Infect Dis. 2010;14(12):e1024–31.
60. Vickerman P, Martin NK, Roy A, Beattie T, Jarlais DD, Strathdee S, et al. Is the HCV-HIV co-infection prevalence amongst injecting drug users a marker for the level of sexual and injection related HIV transmission? Drug Alcohol Depend. 2013;132(1–2):172–81.
61. Wiessing L, Likatavicius G, Hedrich D, Guarita B, van de Laar MJ, Vicente J. Trends in HIV and hepatitis C virus infections among injecting drug users in Europe, 2005 to 2010. Euro Surveill. 2011;16(48):pii: 20031. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20031.
62. Giraudon I, Mounteney J, Kalamara E, Hedrich D. Drug-related infectious diseases in Europe: update from the EMCDDA expert network. Luxembourg: Publications Office of the European Union; 2015. http://www.emcdda.europa.eu/system/files/publications/1815/TD0215722ENN.pdf.
63. Sharma M, Oppenheimer E, Saidel T, Loo V, Garg R. A situation update on HIV epidemics among people who inject drugs and national responses in South-East Asia Region. AIDS. 2009;23(11):1405–13.
64. Horyniak D, Dietze P, Degenhardt L, Higgs P, McIlwraith F, Alati R, et al. The relationship between age and risky injecting behaviours among a sample of Australian people who inject drugs. Drug Alcohol Depend. 2013;132(3):541–6.
65. Burt RD, Thiede H. Reduction in needle sharing among seattle-area injection drug users across 4 surveys, 1994–2013. Am J Public Health. 2016;106(2):301–7.

66. Public Health England, Health Protection Scotland, Public Health Wales, Public Health Agency Northern Ireland. Shooting up: infections among people who inject drugs in the UK, 2014. An update, November 2015. London: Public Health England; 2015. https://webarchive.nationalarchives.gov.uk/20180911151429/https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/475712/Shooting_Up_2015_FINAL.pdf.
67. Lindenburg CE, Krol A, Smit C, Buster MC, Coutinho RA, Prins M. Decline in HIV incidence and injecting, but not in sexual risk behaviour, seen in drug users in Amsterdam: a 19-year prospective cohort study. AIDS. 2006;20(13):1771–5.
68. Fatseas M, Denis C, Serre F, Dubernet J, Daulouede JP, Auriacombe M. Change in HIV-HCV risk-taking behavior and seroprevalence among opiate users seeking treatment over an 11-year period and harm reduction policy. AIDS Behav. 2012;16(7):2082–90.
69. Palmateer NE, Taylor A, Goldberg DJ, Munro A, Aitken C, Shepherd SJ, et al. Rapid decline in HCV incidence among people who inject drugs associated with national scale-up in coverage of a combination of harm reduction interventions. PLoS One. 2014;9(8):e104515.
70. Uuskula A, Raag M, Folch C, Prasad L, Karnite A, van Veen MG, et al. Self-reported testing, HIV status and associated risk behaviours among people who inject drugs in Europe: important differences between East and West. AIDS. 2014;28(11):1657–64.
71. Midgard H, Weir A, Palmateer N, Lo RV III, Pineda JA, Macias J, et al. HCV epidemiology in high-risk groups and the risk of reinfection. J Hepatol. 2016;65(1 Suppl):S33–45.
72. Falster K, Kaldor JM, Maher L. Hepatitis C virus acquisition among injecting drug users: a cohort analysis of a national repeated cross-sectional survey of needle and syringe program attendees in Australia, 1995-2004. J Urban Health. 2009;86(1):106–18.
73. Bluthenthal RN, Kral AH, Gee L, Erringer EA, Edlin BR. The effect of syringe exchange use on high-risk injection drug users: a cohort study. AIDS. 2000;14(5):605–11.
74. Patz JA, Jodrey D. Occupational health in surgery: risks extend beyond the operating room. Aust N Z J Surg. 1995;65(9):627–9.
75. Hagan H. Agent, host, and environment: hepatitis C virus in people who inject drugs. J Infect Dis. 2011;204(12):1819–21.
76. Palmateer NE, Hutchinson SJ, Innes H, Schnier C, Wu O, Goldberg DJ, et al. Review and meta-analysis of the association between self-reported sharing of needles/syringes and hepatitis C virus prevalence and incidence among people who inject drugs in Europe. Int J Drug Policy. 2013;24(2):85–100.
77. Hahn JA, Page-Shafer K, Lum PJ, Bourgois P, Stein E, Evans JL, et al. Hepatitis C virus seroconversion among young injection drug users: relationships and risks. J Infect Dis. 2002;186(11):1558–64.
78. Hagan H, Thiede H, Weiss NS, Hopkins SG, Duchin JS, Alexander ER. Sharing of drug preparation equipment as a risk factor for hepatitis C. Am J Public Health. 2001;91(1):42–6.
79. Heimer R, Binka M, Koester S, Grund JC, Patel A, Paintsil E, et al. Recovery of infectious hepatitis c virus from injection paraphernalia: implications for prevention programs serving people who inject drugs. J Infect Dis. 2018;217(3):466–73.
80. Aaron S, McMahon JM, Milano D, Torres L, Clatts M, Tortu S, et al. Intranasal transmission of hepatitis C virus: virological and clinical evidence. Clin Infect Dis. 2008;47(7):931–4.
81. Muhlberger N, Schwarzer R, Lettmeier B, Sroczynski G, Zeuzem S, Siebert U. HCV-related burden of disease in Europe: a systematic assessment of incidence, prevalence, morbidity, and mortality. BMC Public Health. 2009;9:34.
82. Stanaway JD, Flaxman AD, Naghavi M, Fitzmaurice C, Vos T, Abubakar I, et al. The global burden of viral hepatitis from 1990 to 2013: findings from the Global Burden of Disease Study 2013. Lancet. 2016;388(10049):1081–8.
83. Ly KN, Xing J, Klevens RM, Jiles RB, Ward JW, Holmberg SD. The increasing burden of mortality from viral hepatitis in the United States between 1999 and 2007. Ann Intern Med. 2012;156(4):271–8.

84. Suryaprasad AG, White JZ, Xu F, Eichler BA, Hamilton J, Patel A, et al. Emerging epidemic of hepatitis C virus infections among young nonurban persons who inject drugs in the United States, 2006-2012. Clin Infect Dis. 2014;59(10):1411–9.
85. Smith DJ, Combellick J, Jordan AE, Hagan H. Hepatitis C virus (HCV) disease progression in people who inject drugs (PWID): a systematic review and meta-analysis. Int J Drug Policy. 2015;26(10):911–21.
86. Pierce M, Bird SM, Hickman M, Millar T. National record linkage study of mortality for a large cohort of opioid users ascertained by drug treatment or criminal justice sources in England, 2005–2009. Drug Alcohol Depend. 2015;146:17–23.
87. Matser A, Urbanus A, Geskus R, Kretzschmar M, Xiridou M, Buster M, et al. The effect of hepatitis C treatment and human immunodeficiency virus (HIV) co-infection on the disease burden of hepatitis C among injecting drug users in Amsterdam. Addiction. 2012;107 (3):614–23.
88. Hutchinson SJ, Bird SM, Goldberg DJ. Modeling the current and future disease burden of hepatitis C among injection drug users in Scotland. Hepatology. 2005;42(3):711–23.
89. Hernando V, Perez-Cachafeiro S, Lewden C, Gonzalez J, Segura F, Oteo JA, et al. All-cause and liver-related mortality in HIV positive subjects compared to the general population: differences by HCV co-infection. J Hepatol. 2012;57(4):743–51.
90. Grady B, van den Berg C, van der Helm J, Schinkel J, Coutinho R, Krol A, et al. No impact of hepatitis C virus infection on mortality among drug users during the first decade after seroconversion. Clin Gastroenterol Hepatol. 2011;9(9):786–92.
91. Omland LH, Jepsen P, Weis N, Christensen PB, Laursen AL, Nielsen H, et al. Mortality in HIV-infected injection drug users with active vs cleared hepatitis C virus-infection: a population-based cohort study. J Viral Hepat. 2010;17(4):261–8.
92. ECDC. Hepatitis B and C in the EU neighbourhood: prevalence, burden of disease and screening policies, European centre for disease prevention and control. Stockholm; 2010. https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/TER_100914_Hep_B_C%20_EU_neighbourhood.pdf.
93. Mathers BM, Degenhardt L, Phillips B, Wiessing L, Hickman M, Strathdee SA, et al. Global epidemiology of injecting drug use and HIV among people who inject drugs: a systematic review. Lancet. 2008;372(9651):1733–45.
94. Buning EC, Coutinho RA, van Brussel GH, van Santen GW, van Zadelhoff AW. Preventing AIDS in drug addicts in Amsterdam. Lancet. 1986;1(8495):1435.
95. Hedrich D, Pirona A, Wiessing L. From margin to mainstream: the evolution of harm reduction responses to problem drug use in Europe. Drugs Educ Prev Policy. 2008;15 (6):503–17.
96. van den Berg C, Smit C, van Brussel G, Coutinho R, Prins M. Full participation in harm reduction programmes is associated with decreased risk for human immunodeficiency virus and hepatitis C virus: evidence from the Amsterdam Cohort Studies among drug users. Addiction. 2007;102(9):1454–62.
97. Harm Reduction International. Stone K, editor. The Global State of harm reduction 2016. London: Harm Reduction International; 2016. https://www.hri.global/files/2016/11/14/GSHR2016_14nov.pdf.
98. Larney S, Peacock A, Leung J, Colledge S, Hickman M, Vickerman P, et al. Global, regional, and country-level coverage of interventions to prevent and manage HIV and hepatitis C among people who inject drugs: a systematic review. Lancet Glob Health. 2017;5(12):e1208–20.
99. WHO, UNODC, UNAIDS. WHO, UNODC, UNAIDS technical guide for countries to set targets for universal access to HIV prevention, treatment and care for injecting drug users—2012 revision. Geneva: WHO; 2012. http://apps.who.int/iris/bitstream/10665/77969/1/9789241504379_eng.pdf?ua=1.
100. EMCDDA. European Drug Report 2016. Lisbon: EMCDDA; 2016. http://www.emcdda.europa.eu/system/files/publications/2637/TDAT16001ENN.pdf.

101. Doerrbecker J, Friesland M, Ciesek S, Erichsen TJ, Mateu-Gelabert P, Steinmann J, et al. Inactivation and survival of hepatitis C virus on inanimate surfaces. J Infect Dis. 2011;204 (12):1830–8.
102. Clatts MC, Heimer R, Abdala N, Goldsamt LA, Sotheran JL, Anderson KT, et al. HIV-1 transmission in injection paraphernalia: heating drug solutions may inactivate HIV-1. J Acquir Immune Defic Syndr. 1999;22(2):194–9.
103. Thibault V, Bara JL, Nefau T, Duplessy-Garson C. Hepatitis C transmission in injection drug users: could swabs be the main culprit? J Infect Dis. 2011;204(12):1839–42.
104. Doerrbecker J, Behrendt P, Mateu-Gelabert P, Ciesek S, Riebesehl N, Wilhelm C, et al. Transmission of hepatitis C virus among people who inject drugs: viral stability and association with drug preparation equipment. J Infect Dis. 2013;207(2):281–7.
105. Paintsil E, He H, Peters C, Lindenbach BD, Heimer R. Survival of hepatitis C virus in syringes: implication for transmission among injection drug users. J Infect Dis. 2010;202 (7):984–90.
106. Zule WA, Ticknor-Stellato KM, Desmond DP, Vogtsberger KN. Evaluation of needle and syringe combinations. J Acquir Immune Defic Syndr Hum Retrovirol. 1997;14(3):294–5.
107. Hagan H, Thiede H. Does bleach disinfection of syringes help prevent hepatitis C virus transmission? Epidemiology. 2003;14(5):628–9.
108. Siddharta A, Pfaender S, Malassa A, Doerrbecker J, Anggakusuma, Engelmann M, et al. Inactivation of HCV and HIV by microwave: a novel approach for prevention of virus transmission among people who inject drugs. Sci Rep. 2016;6:36619.
109. Stover HJ, Schaffer D. SMOKE IT! Promoting a change of opiate consumption pattern—from injecting to inhaling. Harm Reduct J. 2014;11:18.
110. Pizzey R, Hunt N. Distributing foil from needle and syringe programmes (NSPs) to promote transitions from heroin injecting to chasing: an evaluation. Harm Reduct J. 2008;5:24.
111. EMCDDA. Health and social responses to drug problems: a European guide. Lisbon: EMCDDA; 2017. http://www.emcdda.europa.eu/system/files/publications/6343/TI_PUBPDF_TD0117699ENN_PDFWEB_20171009153649.pdf.
112. Wiessing L, Klempová D, Hedrich D, Montanari L, Gyarmathy VA. Trends in injecting drug use in Europe. Lisbon: European Monitoring Centre for Drugs and Drug Addiction (EMCDDA); 2010. http://www.emcdda.europa.eu/publications/selected-issues/injecting.
113. ECDC, EMCDDA. Prevention and control of infectious diseases among people who inject drugs. Stockholm: ECDC/EMCDDA; 2011. http://www.emcdda.europa.eu/system/files/publications/638/ECDC-EMCDDA_IDU_guidance_-_web_version_328027.pdf.
114. Hellard M, Sacks-Davis R, Gold J. Hepatitis C treatment for injection drug users: a review of the available evidence. Clin Infect Dis. 2009;49(4):561–73.
115. Edlin BR. Prevention and treatment of hepatitis C in injection drug users. Hepatology. 2002;36 (5 Suppl 1):S210–9.
116. Dalgard O. Follow-up studies of treatment for hepatitis C virus infection among injection drug users. Clin Infect Dis. 2005;40(Suppl 5):S336–8.
117. Bruggmann P. Accessing Hepatitis C patients who are difficult to reach: it is time to overcome barriers. J Viral Hepat. 2012;19(12):829–35.
118. Mehta SH, Lucas GM, Mirel LB, Torbenson M, Higgins Y, Moore RD, et al. Limited effectiveness of antiviral treatment for hepatitis C in an urban HIV clinic. AIDS. 2006;20 (18):2361–9.
119. Mehta SH, Genberg BL, Astemborski J, Kavasery R, Kirk GD, Vlahov D, et al. Limited uptake of hepatitis C treatment among injection drug users. J Community Health. 2008;33 (3):126–33.
120. Aspinall EJ, Corson S, Doyle JS, Grebely J, Hutchinson SJ, Dore GJ, et al. Treatment of hepatitis C virus infection among people who are actively injecting drugs: a systematic review and meta-analysis. Clin Infect Dis. 2013;57(Suppl 2):S80–9.

121. Dimova RB, Zeremski M, Jacobson IM, Hagan H, Des Jarlais DC, Talal AH. Determinants of hepatitis C virus treatment completion and efficacy in drug users assessed by meta-analysis. Clin Infect Dis. 2013;56(6):806–16.
122. Alavi M, Raffa JD, Deans GD, Lai C, Krajden M, Dore GJ, et al. Continued low uptake of treatment for hepatitis C virus infection in a large community-based cohort of inner city residents. Liver Int. 2014;34(8):1198–206.
123. Iversen J, Grebely J, Topp L, Wand H, Dore G, Maher L. Uptake of hepatitis C treatment among people who inject drugs attending Needle and Syringe Programs in Australia, 1999-2011. J Viral Hepat. 2014;21(3):198–207.
124. Martin NK, Foster GR, Vilar J, Ryder S, Cramp ME, Gordon F, et al. HCV treatment rates and sustained viral response among people who inject drugs in seven UK sites: real world results and modelling of treatment impact. J Viral Hepat. 2015;22(4):399–408.
125. Grebely J, Tyndall MW. Management of HCV and HIV infections among people who inject drugs. Curr Opin HIV AIDS. 2011;6(6):501–7.
126. NCHECR. HIV/AIDS, viral hepatitis and sexually transmissible infections in Australia. Annual Surveillance Report 2003. Sydney: National Centre in HIV Epidemiology and Clinical Research (NCHECR), The University of New South Wales; 2003. https://kirby.unsw.edu.au/sites/default/files/kirby/report/SERP_2003-Annual-Surveillance-Report.pdf.
127. Strathdee SA, Latka M, Campbell J, O'Driscoll PT, Golub ET, Kapadia F, et al. Factors associated with interest in initiating treatment for hepatitis C Virus (HCV) infection among young HCV-infected injection drug users. Clin Infect Dis. 2005;40(Suppl 5):S304–12.
128. WHO. Guidelines for the Screening, Care and Treatment of Persons with Hepatitis Infection. Geneva: WHO; 2014. http://apps.who.int/iris/bitstream/10665/111747/1/9789241548755_eng.pdf?ua=1&ua=1.
129. Robaeys G, Grebely J, Mauss S, Bruggmann P, Moussalli J, De GA, et al. Recommendations for the management of hepatitis C virus infection among people who inject drugs. Clin Infect Dis. 2013;57(Suppl 2):S129–37.
130. European Association for the Study of the Liver. EASL recommendations on treatment of hepatitis C 2015. J Hepatol. 2015;63:199–236. https://www.journal-of-hepatology.eu/article/S0168-8278(15)00208-1/fulltext.
131. Barua S, Greenwald R, Grebely J, Dore GJ, Swan T, Taylor LE. Restrictions for medicaid reimbursement of sofosbuvir for the treatment of hepatitis C virus infection in the United States. Ann Intern Med. 2015;163(3):215–23.
132. Lazarus JV, Sperle I, Maticic M, Wiessing L. A systematic review of Hepatitis C virus treatment uptake among people who inject drugs in the European Region. BMC Infect Dis. 2014;14(Suppl 6):S16.
133. Christensen S, Buggisch P, Mauss S, Boker KHW, Schott E, Klinker H, et al. Direct-acting antiviral treatment of chronic HCV-infected patients on opioid substitution therapy: still a concern in clinical practice? Addiction. 2018;113(5):868–82.
134. Hickman M, Martin N, Goldberg D, Fischer G, Duffell E, Simon R. Introduction. In: Hickman M, Martin N, editors. Hepatitis C among drug users in Europe. Lisbon: EMCDDA; 2016. http://www.emcdda.europa.eu/system/files/publications/2953/TDXD16002ENN_final_web.pdf.
135. Martin NK, Vickerman P, Foster GR, Hutchinson SJ, Goldberg DJ, Hickman M. Can antiviral therapy for hepatitis C reduce the prevalence of HCV among injecting drug user populations? A modeling analysis of its prevention utility. J Hepatol. 2011;54(6):1137–44.
136. Martin NK, Vickerman P, Miners A, Foster GR, Hutchinson SJ, Goldberg DJ, et al. Cost-effectiveness of hepatitis C virus antiviral treatment for injection drug user populations. Hepatology. 2012;55(1):49–57.
137. Martin NK, Hickman M, Hutchinson SJ, Goldberg DJ, Vickerman P. Combination interventions to prevent HCV transmission among people who inject drugs: modeling the impact of antiviral treatment, needle and syringe programs, and opiate substitution therapy. Clin Infect Dis. 2013;57(Suppl 2):S39–45.

138. Martin NK, Vickerman P, Grebely J, Hellard M, Hutchinson SJ, Lima VD, et al. Hepatitis C virus treatment for prevention among people who inject drugs: modeling treatment scale-up in the age of direct-acting antivirals. Hepatology. 2013;58(5):1598–609.
139. Martin NK, Vickerman P, Dore GJ, Grebely J, Miners A, Cairns J, et al. Prioritization of HCV treatment in the direct-acting antiviral era: an economic evaluation. J Hepatol. 2016;65 (1):17–25.
140. Martin NK, Vickerman P, Brew IF, Williamson J, Miners A, Irving WL, et al. Is increased hepatitis C virus case-finding combined with current or 8-week to 12-week direct-acting antiviral therapy cost-effective in UK prisons? A prevention benefit analysis. Hepatology. 2016;63(6):1796–808.
141. Martin NK, Hickman M, Vickerman P. Hepatitis C prevention among people who inject drugs: role and impact of hepatitis C treatment, opioid substitution treatment and needle and syringe programmes. Hepatitis C among drug users in Europe. Lisbon: EMCDDA; 2016. http://www.emcdda.europa.eu/system/files/publications/2953/TDXD16002ENN_final_web.pdf.
142. Wood E, Kerr T, Marshall BD, Li K, Zhang R, Hogg RS, et al. Longitudinal community plasma HIV-1 RNA concentrations and incidence of HIV-1 among injecting drug users: prospective cohort study. BMJ. 2009;338:b1649.
143. Miller WC, Powers KA, Smith MK, Cohen MS. Community viral load as a measure for assessment of HIV treatment as prevention. Lancet Infect Dis. 2013;13(5):459–64.
144. Grebely J, Dore GJ. Can hepatitis C virus infection be eradicated in people who inject drugs? Antivir Res. 2014;104:62–72.
145. Hellard M, Doyle JS, Sacks-Davis R, Thompson AJ, McBryde E. Eradication of hepatitis C infection: the importance of targeting people who inject drugs. Hepatology. 2014;59(2):366–9.
146. Hagan LM, Wolpe PR, Schinazi RF. Treatment as prevention and cure towards global eradication of hepatitis C virus. Trends Microbiol. 2013;21(12):625–33.
147. Grebely J, Matthews GV, Lloyd AR, Dore GJ. Elimination of hepatitis C virus infection among people who inject drugs through treatment as prevention: feasibility and future requirements. Clin Infect Dis. 2013;57(7):1014–20.
148. Bruggmann P. Treatment as prevention: the breaking of taboos is required in the fight against hepatitis C among people who inject drugs. Hepatology. 2013;58(5):1523–5.
149. Harris RJ, Thomas B, Griffiths J, Costella A, Chapman R, Ramsay M, et al. Increased uptake and new therapies are needed to avert rising hepatitis C-related end stage liver disease in England: modelling the predicted impact of treatment under different scenarios. J Hepatol. 2014;61(3):530–7.
150. Innes H, Goldberg D, Dillon J, Hutchinson SJ. Strategies for the treatment of Hepatitis C in an era of interferon-free therapies: what public health outcomes do we value most? Gut. 2015;64 (11):1800–9.
151. Harris RJ, Martin NK, Rand E, Mandal S, Mutimer D, Vickerman P, et al. New treatments for hepatitis C virus (HCV): scope for preventing liver disease and HCV transmission in England. J Viral Hepat. 2016;23(8):631–43.
152. Boesecke C, Grint D, Soriano V, Lundgren JD, d'Arminio MA, Mitsura VM, et al. Hepatitis C seroconversions in HIV infection across Europe: which regions and patient groups are affected? Liver Int. 2015;35(11):2384–91.
153. Wandeler G, Schlauri M, Jaquier ME, Rohrbach J, Metzner KJ, Fehr J, et al. Incident hepatitis C virus infections in the Swiss HIV cohort study: changes in treatment uptake and outcomes between 1991 and 2013. Open Forum Infect Dis. 2015;2(1):ofv026.
154. Browne R, Asboe D, Gilleece Y, Atkins M, Mandalia S, Gazzard B, et al. Increased numbers of acute hepatitis C infections in HIV positive homosexual men; is sexual transmission feeding the increase? Sex Transm Infect. 2004;80(4):326–7.
155. Gotz HM, Van DG, Niesters HG, den Hollander JG, Thio HB, De ZO. A cluster of acute hepatitis C virus infection among men who have sex with men—results from contact tracing and public health implications. AIDS. 2005;19(9):969–74.

156. Gambotti L, Batisse D, Colin-de-Verdiere N, Delaroque-Astagneau E, Desenclos JC, Dominguez S, et al. Acute hepatitis C infection in HIV positive men who have sex with men in Paris, France, 2001–2004. Euro Surveill. 2005;10(5):115–7.
157. van de Laar TJ, van der Bij AK, Prins M, Bruisten SM, Brinkman K, Ruys TA, et al. Increase in HCV incidence among men who have sex with men in Amsterdam most likely caused by sexual transmission. J Infect Dis. 2007;196(2):230–8.
158. Danta M, Brown D, Bhagani S, Pybus OG, Sabin CA, Nelson M, et al. Recent epidemic of acute hepatitis C virus in HIV-positive men who have sex with men linked to high-risk sexual behaviours. AIDS. 2007;21(8):983–91.
159. Serpaggi J, Chaix ML, Batisse D, Dupont C, Vallet-Pichard A, Fontaine H, et al. Sexually transmitted acute infection with a clustered genotype 4 hepatitis C virus in HIV-1-infected men and inefficacy of early antiviral therapy. AIDS. 2006;20(2):233–40.
160. Fierer DS, Uriel AJ, Carriero DC, Klepper A, Dieterich DT, Mullen MP, et al. Liver fibrosis during an outbreak of acute hepatitis C virus infection in HIV-infected men: a prospective cohort study. J Infect Dis. 2008;198(5):683–6.
161. Luetkemeyer A, Hare CB, Stansell J, Tien PC, Charlesbois E, Lum P, et al. Clinical presentation and course of acute hepatitis C infection in HIV-infected patients. J Acquir Immune Defic Syndr. 2006;41(1):31–6.
162. Holubar M, Taylor L, Wu K, Bosch R, Mayer K, Tashima K. Hepatitis C virus (HCV) antibody seroconversion in a US. HIV-infected male clinical trials population. In: 60th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), Boston, MA, USA, October/November 2009. Abstract LB14.
163. Matthews GV, Hellard M, Kaldor J, Lloyd A, Dore GJ. Further evidence of HCV sexual transmission among HIV-positive men who have sex with men: response to Danta et al. AIDS. 2007;21(15):2112–3.
164. Urbanus AT, van de Laar TJ, Stolte IG, Schinkel J, Heijman T, Coutinho RA, et al. Hepatitis C virus infections among HIV-infected men who have sex with men: an expanding epidemic. AIDS. 2009;23(12):F1–7.
165. Ingiliz P, Martin TC, Rodger A, Stellbrink HJ, Mauss S, Boesecke C, et al. HCV reinfection incidence and spontaneous clearance rates in HIV-positive men who have sex with men in Western Europe. J Hepatol. 2017;66(2):282–7.
166. Hoornenborg E, Achterbergh RCA, Schim Van Der Loeff MF, Davidovich U, Hogewoning A, Vries HJC, et al. Men who have sex with men starting pre-exposure prophylaxis (PrEP) are at risk of HCV infection: evidence from the Amsterdam PrEP study. AIDS. 2017;31:1.
167. Hagan H, Jordan AE, Neurer J, Cleland CM. Incidence of sexually transmitted hepatitis C virus infection in HIV-positive men who have sex with men. AIDS. 2015;29(17):2335–45.
168. Ghosn J, Deveau C, Goujard C, Garrigue I, Saichi N, Galimand J, et al. Increase in hepatitis C virus incidence in HIV-1-infected patients followed up since primary infection. Sex Transm Infect. 2006;82(6):458–60.
169. Giraudon I, Ruf M, Maguire H, Charlett A, Ncube F, Turner J, et al. Increase in diagnosed newly acquired hepatitis C in HIV-positive men who have sex with men across London and Brighton, 2002-2006: is this an outbreak? Sex Transm Infect. 2008;84(2):111–5.
170. Wandeler G, Gsponer T, Bregenzer A, Gunthard HF, Clerc O, Calmy A, et al. Hepatitis C virus infections in the Swiss HIV Cohort Study: a rapidly evolving epidemic. Clin Infect Dis. 2012;55(10):1408–16.
171. Stellbrink HJ, Schewe CK, Vogel M, Hoffmann C, Noah C. Incidence, Genotype Distribution, and Prognosis of Sexually Transmitted Acute Hepatitis C in a Cohort of HIV-infected Patients, Abstract 645. 17th Conference on Retroviruses and Opportunistic Infections (CROI 2010). San Francisco, USA. 2010. https://goo.gl/NFo7v6.
172. Boerekamps A, Van den Berk GE, Fanny LN, Leyten EM, Van Kasteren ME, Van EA, et al. Declining HCV incidence in Dutch HIV positive men who have sex with men after unrestricted access to HCV therapy. Clin Infect Dis. 2018;66(9):1360–5.

173. Vanhommerig JW, Stolte IG, Lambers FA, Geskus RB, van de Laar TJ, Bruisten SM, et al. Stabilizing incidence of hepatitis C virus infection among men who have sex with men in Amsterdam. J Acquir Immune Defic Syndr. 2014;66(5):e111–5.
174. Pradat P, Huleux T, Raffi F, Delobel P, Valantin MA, Poizot-Martin I, et al. Incidence of new hepatitis C virus infection is still increasing in French MSM living with HIV. AIDS. 2018;32 (8):1077–82.
175. Witt MD, Seaberg EC, Darilay A, Young S, Badri S, Rinaldo CR, et al. Incident hepatitis C virus infection in men who have sex with men: a prospective cohort analysis, 1984-2011. Clin Infect Dis. 2013;57(1):77–84.
176. Nishijima T, Shimbo T, Komatsu H, Hamada Y, Gatanaga H, Oka S. Incidence and risk factors for incident Hepatitis C infection among men who have sex with men with HIV-1 infection in a large Urban HIV clinic in Tokyo. J Acquir Immune Defic Syndr. 2014;65(2):213–7.
177. Martin TC, Martin NK, Hickman M, Vickerman P, Page EE, Everett R, et al. Hepatitis C virus reinfection incidence and treatment outcome among HIV-positive MSM. AIDS. 2013;27 (16):2551–7.
178. Lambers FA, Prins M, Thomas X, Molenkamp R, Kwa D, Brinkman K, et al. Alarming incidence of hepatitis C virus re-infection after treatment of sexually acquired acute hepatitis C virus infection in HIV-infected MSM. AIDS. 2011;25(17):F21–7.
179. van de Laar T, Pybus O, Bruisten S, Brown D, Nelson M, Bhagani S, et al. Evidence of a large, international network of HCV transmission in HIV-positive men who have sex with men. Gastroenterology. 2009;136(5):1609–17.
180. McFarlane M, Bull SS, Rietmeijer CA. The Internet as a newly emerging risk environment for sexually transmitted diseases. JAMA. 2000;284(4):443–6.
181. Schmidt AJ, Rockstroh JK, Vogel M, An der Heiden M, Baillot A, Krznaric I, et al. Trouble with bleeding: risk factors for acute hepatitis C among HIV-positive gay men from Germany—a case-control study. PLoS One. 2011;6(3):e17781.
182. Lemoine M, Thursz M. Hepatitis C, a global issue: access to care and new therapeutic and preventive approaches in resource-constrained areas. Semin Liver Dis. 2014;34(1):89–97.
183. Hauri AM, Armstrong GL, Hutin YJ. The global burden of disease attributable to contaminated injections given in health care settings. Int J STD AIDS. 2004;15(1):7–16.
184. Brouard C, Pradat P, Delarocque-Astagneau E, Silvain C. Epidemiological characteristics and medical follow-up of 61 patients with acute hepatitis C identified through the hepatitis C surveillance system in France. Epidemiol Infect. 2008;136(7):988–96.
185. Delarocque-Astagneau E, Baffoy N, Thiers V, Simon N, De VH, Laperche S, et al. Outbreak of hepatitis C virus infection in a hemodialysis unit: potential transmission by the hemodialysis machine? Infect Control Hosp Epidemiol. 2002;23(6):328–34.
186. Bronowicki JP, Venard V, Botte C, Monhoven N, Gastin I, Chone L, et al. Patient-to-patient transmission of hepatitis C virus during colonoscopy. N Engl J Med. 1997;337(4):237–40.
187. Delarocque-Astagneau E, Pillonel J, de Valk H, Perra A, Laperche S, Desenclos JC. Modes of hepatitis C virus transmission: methodological approaches. Rev Epidemiol Sante Publique 2006;54 Spec No 1:1S5–1S14.
188. Mele A, Spada E, Sagliocca L, Ragni P, Tosti ME, Gallo G, et al. Risk of parenterally transmitted hepatitis following exposure to surgery or other invasive procedures: results from the hepatitis surveillance system in Italy. J Hepatol. 2001;35(2):284–9.
189. Bruggmann P, Berg T, Ovrehus AL, Moreno C, Brandao Mello CE, Roudot-Thoraval F, et al. Historical epidemiology of hepatitis C virus (HCV) in selected countries. J Viral Hepat. 2014;21(Suppl 1):5–33.
190. Falla AM, Ahmad AA, Duffell E, Noori T, Veldhuijzen IK. Estimating the scale of chronic hepatitis C virus infection in the EU/EEA: a focus on migrants from anti-HCV endemic countries. BMC Infect Dis. 2018;18(1):42.
191. Falade-Nwulia O, Sulkowski MS, Merkow A, Latkin C, Mehta SH. Understanding and addressing hepatitis C reinfection in the oral direct-acting antiviral era. J Viral Hepat. 2018;25(3):220–7.

192. Sacks-Davis R, Grebely J, Dore GJ, Osburn W, Cox AL, Rice TM, et al. Hepatitis C virus reinfection and spontaneous clearance of reinfection–the InC3 study. J Infect Dis. 2015;212 (9):1407–19.
193. Ingiliz P, Krznaric I, Stellbrink HJ, Knecht G, Lutz T, Noah C, et al. Multiple hepatitis C virus (HCV) reinfections in HIV-positive men who have sex with men: no influence of HCV genotype switch or interleukin-28B genotype on spontaneous clearance. HIV Med. 2014;15 (6):355–61.
194. Hill AM, Simmons B, Saleem J, Cooke G. Five-year risk of late relapse or reinfection with hepatitis C after sustained virological response: meta-analysis of 49 studies in 8534 Patients. Abstract 654. Conference on Retroviruses and Opportunistic Infections (CROI) . 2015. https://www.croiconference.org/abstract/five-year-risk-late-relapse-or-reinfection-hepatitis-c-after-sustained-virological-response/.
195. Simmons B, Saleem J, Hill A, Riley RD, Cooke GS. Risk of late relapse or reinfection with hepatitis C virus after achieving a sustained virological response: a systematic review and meta-analysis. Clin Infect Dis. 2016;62(6):683–94.
196. Young J, Rossi C, Gill J, Walmsley S, Cooper C, Cox J, et al. Risk factors for hepatitis C virus reinfection after sustained virologic response in patients coinfected with HIV. Clin Infect Dis. 2017;64(9):1154–62.
197. Sulkowski MS, Eron JJ, Wyles D, Trinh R, Lalezari J, Wang C, et al. Ombitasvir, paritaprevir co-dosed with ritonavir, dasabuvir, and ribavirin for hepatitis C in patients co-infected with HIV-1: a randomized trial. JAMA. 2015;313(12):1223–31.
198. Rockstroh JK, Nelson M, Katlama C, Lalezari J, Mallolas J, Bloch M, et al. Efficacy and safety of grazoprevir (MK-5172) and elbasvir (MK-8742) in patients with hepatitis C virus and HIV co-infection (C-EDGE CO-INFECTION): a non-randomised, open-label trial. Lancet HIV. 2015;2(8):e319–27.
199. Sarrazin C, Isakov V, Svarovskaia E, Martin R, Chodavarapu K, Hedskog C, et al. HCV reinfection in phase 3 studies of sofosbuvir. Abstract P0063. 50th Annual Meeting of the European Association for the Study of the Liver. 2014;Vienna. https://natap.org/2015/EASL/EASL_78.htm.
200. Hatzakis A, Wait S, Bruix J, Buti M, Carballo M, Cavaleri M, et al. The state of hepatitis B and C in Europe: report from the hepatitis B and C summit conference*. J Viral Hepat. 2011;18 (Suppl 1):1–16.
201. Thomas DL. Curing hepatitis C with pills: a step toward global control. Lancet. 2010;376 (9751):1441–2.
202. WHO. Guidelines on hepatitis B and C testing. Geneva: World Health Organisation; 2017. https://goo.gl/UyALs7.
203. Zhou K, Fitzpatrick T, Walsh N, Kim JY, Chou R, Lackey M, et al. Interventions to optimise the care continuum for chronic viral hepatitis: a systematic review and meta-analyses. Lancet Infect Dis. 2016;16(12):1409–22.
204. Martinello M, Hajarizadeh B, Grebely J, Dore GJ, Matthews GV. HCV cure and reinfection among people with HIV/HCV coinfection and people who inject drugs. Curr HIV/AIDS Rep. 2017;14(3):110–21.
205. Wright N, Reimer J, Somaini L, Roncero C, Maremmani I, Simon N, et al. Are we ready to treat hepatitis C virus in individuals with opioid use disorder: assessment of readiness in European countries on the basis of an expert-generated model. Eur J Gastroenterol Hepatol. 2017;29(11):1206–14.
206. Martin NK, Thornton A, Hickman M, Sabin C, Nelson M, Cooke GS, et al. Can hepatitis C virus (HCV) direct-acting antiviral treatment as prevention reverse the HCV epidemic among men who have sex with men in the United Kingdom? Epidemiological and modeling insights. Clin Infect Dis. 2016;62(9):1072–80.
207. Martin NK, Hickman M, Nelson M, Thornton A, Sabin C, Lattimore S, et al. Understanding and preventing the HCV epidemic among men who have sex with men in the UK: a mathematical modelling analysis. J Hepatol. 2015;62(S2):926–31. https://www.researchgate.

net/publication/279230783_P1289_Understanding_and_preventing_the_HCV_epidemic_among_men_who_have_sex_with_men_in_the_UK_A_mathematical_modelling_analysis.

208. Ingiliz P, Boyd A, Steininger K, Caranico A, Dupke S, Krznaric I, et al. Long-term follow-up of HIV-positive men who have sex with men (MSM) with acute hepatitis C virus (HCV) infection: high rates of treatment and low rates of liver-related complications. abstract 671. Conference on Retroviruses and Opportunistic Infections (CROI). 2015. https://goo.gl/uLFhW8.
209. Harris M, Rhodes T. Scaling up hepatitis C treatment: taking into account the needs and perspectives of people who inject drugs. Hepatitis C among drug users in Europe. Lisbon: EMCDDA; 2016. http://www.emcdda.europa.eu/system/files/publications/2953/TDXD16002ENN_final_web.pdf.

Natural History of Hepatitis C Infection

7

Elisabetta Degasperi and Massimo Colombo

7.1 Introduction

Natural history of hepatitis C virus (HCV) infection has been extensively studied in the past, since for several decades, scarcity of potent and effective antiviral treatments allowed observational studies of large untreated cohorts. However, deep understanding of HCV natural history has been always hampered by the asymptomatic course of the disease, both in the acute and in the chronic infection phase. As a consequence, many studies conducted in the field of natural history have prospectively evaluated only selected patients' cohorts according to presence of HCV risk factors (blood donors, post-transfusion HCV cohorts) that could maximize chance of early diagnosing HCV infection, thus generating data about HCV natural course only in peculiar patient subsets. Less selected data mainly come from retrospective or retrospective-prospective studies where a precise estimate of HCV infection could be done according to patient's medical history. However, also these data have often been generated in secondary or tertiary centers, so introducing a referral bias as patients referred for treatment were often those with a progressive disease. Despite all these caveats, the natural course of HCV has been extensively characterized both in the acute and in the chronic infection leading to liver disease and its complications [1].

E. Degasperi
Division of Gastroenterology and Hepatology, Fondazione IRCCS CA' Granda Ospedale Maggiore Policlinico, Milan, Italy
e-mail: elisabetta.degasperi@policlinico.mi.it

M. Colombo (✉)
Liver Center San Raffaele Hospital, Milan, Italy
e-mail: mcolombo46@yahoo.it

A. Hatzakis (ed.), *Hepatitis C: Epidemiology, Prevention and Elimination*,
https://doi.org/10.1007/978-3-030-64649-3_7

7.2 Acute HCV Infection

HCV infection is acquired through parenteral transmission; risk factors include transfusion of blood products and medical/surgical procedures before 1990–1992 (when anti-HCV screening was introduced), intravenous drug use, tattooing and piercing, and at-risk sexual intercourse (especially MSM—men who have sex with men), while vertical (mother-to-child) transmission rarely occurs [1, 2].

HCV-RNA becomes detectable 7–21 days after infection, while aminotransferases (ALT) increase after 4–12 weeks. Acute infection is asymptomatic in 70–80% of patients, while only 20–30% develop non-specific flu-like symptoms or overt hepatitis with jaundice. Anti-HCV antibodies become detectable 1–3 months after exposure (that usually is also the onset of clinical symptoms); however, time to seroconversion can vary within 20–250 days, and anti-HCV are undetectable in immunocompromised patients, so that diagnosis of acute HCV infection still relies on serum HCV-RNA assessment. Even in case of symptomatic HCV infection, acute hepatitis is characterized by good clinical outcome, and fulminant hepatitis is rare. Spontaneous HCV clearance occurs only in 20–30% of patients and is characterized by progressive ALT and HCV-RNA decline till HCV-RNA undetectability usually 3–4 months after acute infection, while a fluctuating pattern of ALT and HCV-RNA can be observed in 15% of patients before self-limiting infection. In 70–80% of cases, however, HCV-RNA persists detectable, so defining transition to chronic hepatitis infection 6 months after onset [3–7]. As the turning point from acute self-limiting to persistent HCV infection has been identified approximately at 3–4 months after onset in symptomatic patients, antiviral treatment for the acute infection phase should be started in patients where HCV-RNA tends to persist after this time period.

Several factors have been associated with spontaneous HCV clearance, including age, gender, symptomatic acute infection, immune response, and, more recently, genetic factors [8–10].

Younger age at infection has been associated with increased rates of spontaneous HCV clearance, as well as female gender, where a key role of estrogen hormones has been hypothesized [10, 11]. Symptomatic acute infection has been also recognized as a predictive factor of spontaneous HCV clearance [12, 13].

Effective adaptive and specific CD4 and CD8 T-cell immune responses are required to clear viral infections [14–16]; HCV has been shown to perturb adaptive immune responses through structural (E2, core) and non-structural (NS3, NS5A) HCV proteins, by interfering with Toll-like receptor signaling [17]. HCV infection is also associated with disruption of specific immunity through activation of T regulatory cells, T-cell apoptosis, and Th1 to Th2 shift of immune responses [18, 19]. Some studies have also concentrated on natural killer cells and their role in HCV clearance, although other studies have shown HCV interference also with cytotoxic NK activity [20, 21].

More recently, the single nucleotide polymorphism (SNP) rs12979860 near the interleukin 28B (IL28B) region, initially identified as the strongest baseline genetic predictor of sustained virologic response (SVR) to antiviral treatment, was also

associated with spontaneous HCV clearance [22–25]. Homozygosity for the C allele has been associated with the highest chances of spontaneous clearance, where highest prevalence of the CC genotype is found in the East Asian countries and can eventually explain good HCV outcomes observed in these world areas.

7.3 Chronic HCV Infection

7.3.1 Fibrosis Progression in Chronic HCV Infection

Chronic HCV infection is characterized by persistent hepatic inflammation leading to progressive fibrosis deposition in the liver, eventually resulting in cirrhosis development and its complications. Many studies have attempted to estimate fibrosis progression in chronic HCV infection and describe this complex process that is influenced by many host, viral, and environmental factors, resulting in different fibrosis progression rates at the individual level. Two approaches have been mainly used to depict fibrosis progression: the first relies upon serial liver biopsies and calculates fibrosis progression rate by considering time intervals between each fibrosis assessment (direct approach), while the second approach derives fibrosis progression from a single liver biopsy basing on estimated time of HCV infection in patient's medical history (indirect approach). Although liver biopsy still represents the gold standard to assess fibrosis stage, all approaches can be influenced by the possibility of sampling error and potential misclassification of correct fibrosis stage due to inadequate liver sampling, irregular fibrosis deposition, and intra-/interobserver variability by liver pathologists [26].

A direct approach with repeated liver biopsies has the advantage to calculate transition rates for any fibrosis stage and to rely upon several assessments of liver biopsy. However, very few studies investigated fibrosis progression rate with this approach: in 2003, Ghany and colleagues evaluated fibrosis progression rate in a cohort of 123 HCV untreated patients who underwent two liver biopsies at a mean interval of 44 months (range 4–212 months). 48 out of 123 (39%) patients showed fibrosis progression: 75% had a 1-point increase in Ishak score, 25% a $\geq$2-point increase, and 9% progressed to cirrhosis. Overall progression rate was 0.12 fibrosis units/year, predicting cirrhosis development in 50 years, given a linear fibrosis progression. However, the authors find that ALT levels, older age, and inflammation degree in initial liver biopsy were predictors of accelerated fibrosis progression [27]. The following year, Ryder and colleagues partially replicated these findings in 214 HCV untreated patients with mild baseline fibrosis (188/214 patients with fibrosis stage 0–1 according to Ishak score), prospectively followed up with repeated liver biopsies at a median 2.5-year intervals: 70/219 (33%) patients showed progression of at least 1 fibrosis point, while 23 patients (11%) progressed at least 2 points over a median follow-up of 30 months. Independent predictors of fibrosis progression were age and fibrosis stage at first biopsy [28].

On the other hand, most literature studies concerning fibrosis progression have been conducted with the indirect approach that is inferring fibrosis progression rate

from a single liver biopsy and disease duration, according to estimated time of HCV infection. The landmark study in this field was conducted in 1997 by Poynard and colleagues on 2235 HCV untreated patients from 3 different French cohorts and 1 available liver biopsy staged according to the METAVIR score. Fibrosis progression rate was calculated as a ratio between fibrosis stage and duration of infection according to patient's medical history: median fibrosis progression rate resulted 0.133 fibrosis units/year (95% CI 0.125–0.143) with a median time from infection to cirrhosis progression of 30 years (range 28–32). However, the analysis led to identify 3 different patient groups according to different observed patterns of fibrosis progression: indeed 377 (33%) patients had an expected time to cirrhosis less than 20 years (fast progressors), 356 (31%) did not show any expected progression to cirrhosis over 50 years (slow progressors), and the remaining were intermediate progressors. Three independent factors were associated with increased rates of fibrosis progression: age at infection, >50 g/day alcohol intake, and male sex, where time to cirrhosis progression ranged from 13 years in men infected after the age of 40–42 years in women not drinking alcohol and infected before age of 40 [29].

Since this pivotal study, many other papers have been published about fibrosis progression, leading to highly heterogeneous results, mainly due to study design, patient population studied (especially concerning prevalence of known co-factors of fibrosis progression), and methods used to estimate fibrosis progression rate. Globally considered, development of cirrhosis is reported in approximately 10–20% of patients following 20–30 years of chronic infection, prevalence of cirrhosis ranging from 2–3% to 51% according to different studies. Indeed retrospective data derived from tertiary referrals centers have often reported higher progression rates to cirrhosis (17–55% at 20 years) [1, 30–33], compared to prospective data coming from blood donors and community cohorts, where the corresponding figures were 1–2% cirrhosis development after 20 years [34–36]. This can be easily explained by presence of ascertainment bias, since patients coming from clinical settings and tertiary liver centers are more likely to suffer from advanced disease stages, as this was the indication for considering antiviral treatment and referring the patient to a more specialized center. A recent meta-analysis of 111 studies about a total of 33.121 HCV patients reported an overall 16% estimated prevalence of cirrhosis at 20 years and 41% at 30 years, confirming lower rates in retrospective-prospective studies and higher cirrhosis rates in clinical settings and cross-sectional studies from liver referral centers. Annual mean stage-specific transition probabilities calculated by Markov maximum likelihood estimation method were 0.117 (stage F0–F1 according to METAVIR score), 0.085 (F1–F2), 0.120 (F2–F3), and 0.116 (F3–F4). According to this model, as previously reported in other studies, fibrosis does not follow a linear progression, rather a nonlinear upward curve with an accelerated progression with older age and duration of infection [37].

7.3.2 Co-factors Affecting Fibrosis Progression

Several host, viral, and environmental factors have been shown to influence natural history of chronic hepatitis C, making fibrosis progression highly variable at the individual level: age, gender, ethnicity, genetic background, viral genotype, ALT levels, human immunodeficiency virus (HIV)/hepatitis B virus (HBV) coinfection, alcohol consumption, obesity, and insulin resistance.

Natural history of HCV infection according to presence of co-factors is depicted in Fig. 7.1.

7.3.2.1 Age

Age at the time of HCV infection has been shown to significantly affect fibrosis progression during HCV natural history: indeed there is a positive correlation between age at infection and development of advanced fibrosis, so that patients infected during childhood usually show mild and non-progressive diseases. This has been extensively confirmed in many studies focusing on transfusional and surgical cohorts; a German study comparing children undergoing cardiac surgery with age and sex-matched controls found only 3/67 (4%) patients developing progressive liver disease over 20 years of infection [38]. On the other hand, age at infection above 40 years was independently associated with increased rates of fibrosis progression by the Poynard study conducted on 2235 patients [29] and confirmed by other studies [29, 39, 40]. Fibrosis progression seems also to accelerate overtime with an exponential rate in older ages, although it is difficult to evaluate fibrosis progression by age independently from other host and environmental factors. The mechanisms linking aging to accelerated fibrosis progression are not still

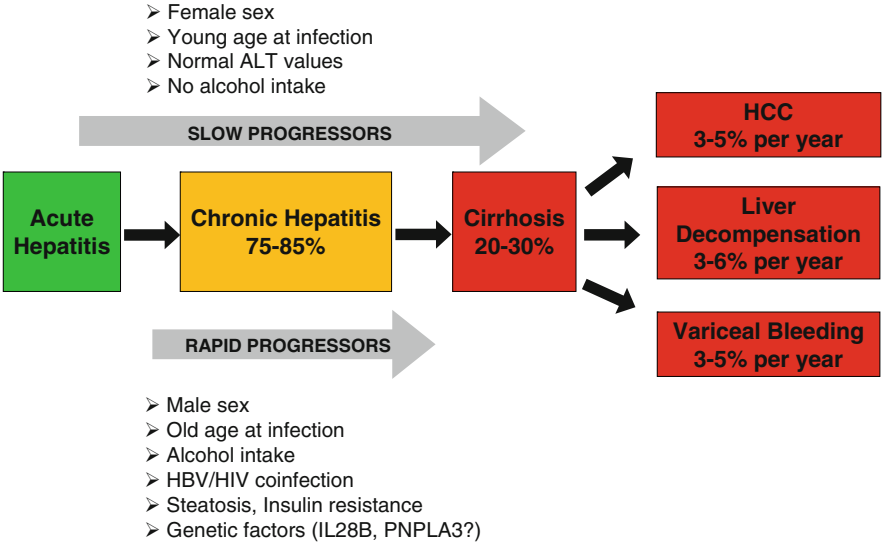


Fig. 7.1 Natural history of HCV infection according to co-factors of disease progression

understood; however, impaired regenerative capacity, cellular senescence by telomere shortening, and interplay with immune responses could play an important role.

7.3.2.2 Gender

Male sex has been associated with accelerated fibrosis progression, with a more than 30% increased annual fibrosis progression rates compared to female sex (0.154 vs. 0.111 fibrosis units/year, $p < 0.001$) [29]. Many studies conducted on female cohorts have confirmed the protective role of female gender, as two prospective studies in 376 Irish and 529 German women infected by contaminated anti-D immunoglobulin showed 0.5% and 2% cirrhosis development after 17 and 25 years of HCV infection, respectively [33, 34]. A subsequent update of the German cohort reported 9% cirrhosis after 35 years of chronic infection [41]. Estrogen hormones seem to be the main responsible for the protective role of female gender, as estradiol has been showed to inhibit stellate cells which are involved in fibrosis deposition in the liver [42, 43]. Consistently with these observations, a retrospective study including 710 HCV-infected women found a significant association between postmenopausal state and more advanced liver fibrosis, meaning that fibrosis progression accelerates in parallel with estrogen deprivation [44]. This was also confirmed in another large retrospective analysis on 472 women, where patients receiving postmenopausal hormone replacement therapy showed lower rates of fibrosis progression compared to untreated women [45].

7.3.2.3 Ethnicity

African-American patients show higher rates of advanced fibrosis, hepatocellular carcinoma (HCC), and liver-related mortality when compared to Caucasians [46, 47], despite some authors hypothesized that this could result also from limited care access in this patient group [48]. Indeed a more recent retrospective study on 812 HCV patients with available liver biopsies found the highest cirrhosis rates in Hispanic patients with respect to other groups, as cirrhosis was diagnosed in 78/157 (50%) Hispanic patients vs. 134/354 (38%) non-Hispanic white and only 72/301 (24%) African-Americans ($p < 0.001$). Cirrhosis was also associated with older age, longer duration of infection, body mass index (BMI), alcohol consumption, and diabetes, while at multivariate analysis, only BMI and ethnicity remained significant [49].

Concerning Eastern ethnicity, a study conducted in the UK evaluated natural history of HCV infection in 120 patients of Indian origin compared to 2123 white patients, reporting overall higher rates of advanced fibrosis in Asian patients. However, when adjusting data for disease duration in patients with a known date of infection, fibrosis progression resulted similar in the two groups [50].

7.3.2.4 Genetic Background

Several studies tried to identify genetic determinants of accelerated fibrosis progression in chronic HCV infection, by concentrating on HLA major histocompatibility complex, genes involved in extracellular matrix turnover and fibrosis deposition, as well as key steps of inflammatory pathways. Class II alleles DRB*0405 and

DQB1*0401 of HLA major histocompatibility complex have been associated with fibrosis progression, whereas other alleles such as DRB1*11 and DQB1*03 have shown a protective role [32, 51, 52]. Liver fibrosis has been also associated with upregulation of 11 genes including extracellular matrix production/remodeling factors (TIMP1, MMP7), growth factor receptors (CCR2, CXCR3, CXCR4), and cytokines (CXCL6, IL-8, IL-2), where this genetic signature showed good accuracy in discriminating mild versus moderate fibrosis [53]. More recently, the availability of genome-wide association studies allowed extensive testing of multiple gene regions and SNPs in large patient cohorts, in order to identify significant association with advanced fibrosis. A French cohort study evaluating 2342 HCV patients of European descent identified a significant association between fibrosis progression and SNPs rs16851720 and rs4374383, whose encoded products are involved in antioxidant and apoptotic processes [54]. In the Swiss Hepatitis C Cohort Study, conducted on 1461 HCV patients with available liver biopsy and estimated date of infection, age at infection, sex, and SNPs rs9380516 (TULP1), rs738409 (PNPLA3), rs4374383 (MERTK), and rs910049 (major histocompatibility complex region) were associated with higher fibrosis progression rates. Results were replicated in three additional independent cohorts and a following meta-analysis [55].

Since the identification of the SNP rs12979860 near the interleukin 28B gene as the strongest predictor of SVR to peginterferon (PegIFN) + ribavirin (Rbv) antiviral therapy, many studies tried to investigate a potential role of IL28B genotype in HCV natural history, with conflicting results: if some authors found a significant association between the IL28B T unfavorable allele and fibrosis progression, other studies did not replicate these results [56, 57]. Finally, much research has also focused on the rs738409 C > G SNP in the patatin-like phospholipase domain-containing protein 3 (PNPLA3), which is a recognized genetic determinant of liver steatosis and fibrosis in non-alcoholic fatty liver disease (NAFLD): the mutated PNPLA3 GG genotype has been associated with liver damage in terms of steatosis and increased fibrosis also in patients with chronic HCV infection by two large studies evaluating a total of 1356 HCV patients [58, 59].

7.3.2.5 Viral Genotype

The influence of HCV genotype on fibrosis progression has been much debated, as many studies in the past decades reported a more aggressive disease pattern with accelerated fibrosis progression and increased HCC risk in genotype 1b patients [60–64]. However, other authors did not replicate these findings, as influence of HCV genotype was no longer significant after adjusting data for duration of infection and patient age, probably meaning that the proposed role of HCV genotype 1b was related to a selection bias of patients with longer disease duration [65–67]. Moreover, reduced chances of HCV cure by PegIFN + Rbv treatment could also account for a worse prognosis in HCV-1b patients when compared with other genotypes with increased SVR rates.

HCV genotype 2 has been also claimed responsible for accelerated liver disease progression due to the possibility of ALT flares that seem to occur more frequently in HCV-2 compared to other genotypes [68, 69]. Genotype 3 has been found associated

with development of liver steatosis due to a pro-steatogenic effect of HCV-3 core protein [70, 71]. More recently, a large study from the Swiss Hepatitis C Cohort including 1189 patients with date of infection reported HCV-3 as an independent risk factor for accelerated fibrosis progression (OR 1.89, CI 1.37–2.61, $p < 0.001$) together with male sex, age at infection, and histologic activity [72, 73]. In addition to a potential role of HCV-3 genotype in fibrosis progression per se, it has to be considered that infection with this genotype is epidemiologically linked to at-risk behaviors such as intravenous drug abuse and alcohol intake that could also contribute to a more aggressive disease.

7.3.2.6 ALT Values

Patients with persistently normal ALT values seem to display a slowly progressive liver disease compared to abnormal or fluctuating ALT patterns [74–76]. This is not surprising as biochemical values are a surrogate markers of histologic intrahepatic inflammation, which emerges in many studies as a determinant of liver fibrosis progression [31, 77]. However, also patients with persistently normal ALT values can display fibrosis progression, and up to 10% of patients have bridging fibrosis at liver biopsy, thus suggesting that ALT values are not reliable markers of disease severity [76, 78].

7.3.2.7 HIV and HBV Coinfection

Prevalence of HBV/HCV co-infection is estimated approximately 5–20% in HBsAg + patients and 2–10% in HCV patients, as both viruses share common transmission routes and risk factors [79, 80]. HBV and HCV reciprocally interfere leading to multiple possible patterns of viral replication: indeed, most frequently HCV actively replicates over HBV, whereas active HBV replication can prevail on HCV, and also both active viral replication can occur [79]. Although long-term prospective studies are lacking, the outcome of HBV/HCV co-infection is considered more severe than mono-infection, as data suggest higher rates of cirrhosis, liver decompensation, and HCC development in co-infected patients [81–85]. However, a recent meta-analysis showed contradictory results concerning increased HCC risk in these patients [86].

Also HIV and HCV infections share common risk factors, where HCV prevalence is strictly linked to at-risk behaviors such as intravenous drug abuse and risky sexual intercourse. In HIV-infected patients, HCV co-infection has been associated with higher HCV-RNA serum levels and is an established risk factor for liver disease severity and progression to advanced fibrosis and liver-related events [87–89]. HIV co-infection was also a negative predictors of response to PegIFN + Rbv antiviral treatment, although the development of direct-acting antiviral drugs for HCV therapy has now challenged this issue and cure rates for mono or co-infected patients are now largely coincident.

7.3.2.8 Alcohol Intake

Despite many clinical data have been generated concerning influence of alcohol intake on HCV natural history, results are still highly heterogeneous. One of the main issues in evaluating alcohol as a co-factor affecting fibrosis progression is the

poor standardization of alcohol intake as self-reported information, although many specific questionnaires have been developed in order to improve objectivity. In addition, the concept of "standard drink" unit, defined as the beverage dose containing a fixed alcohol quantity (100 mL wine = 1 bottle beer = 1 shot of spirits), has been widely adopted to obtain reproducible data, although quantity of alcohol contained in a standard drink can vary according to different geographical areas (i.e., 10 g/alcohol in Europe, 12 g in the USA, and 23 g in Japan) [91, 92].

Many studies have consistently demonstrated that alcohol accelerates fibrosis progression in HCV, although many different cut-off values for the risky alcohol dose have been proposed: in the French study by Poynard and colleagues conducted on 2235 HCV patients, a daily alcohol intake of 50 g/day was associated with more advanced fibrosis development, independently from patient age and HCV infection duration, conferring a 34% increase in fibrosis progression rate compared to patients drinking less than 50 g/day [29]. Other studies have suggested an alcohol-related damage in HCV patients also for minor levels of alcohol intake: another French study evaluating 260 HCV patients with available liver biopsy has demonstrated a direct proportional increase between hepatic inflammation at liver biopsy and alcohol intake starting since a daily dose of 20 g/day. Alcohol intake was associated with moderate-severe steatosis (54% in patients drinking 31–50 g/day vs. 26% in patients drinking <20 g/day) and moderate-severe fibrosis (67% in 31–50 g/day vs. 38% in 20–30 g/day). A moderate alcohol intake defined as 31–50 g/day was confirmed significantly associated with fibrosis progression at multivariate analysis [93]. More recently, a Scottish group has tried to determine the alcohol-attributable risk for cirrhosis in HCV-related liver disease. In 1620 patients with known date of infection, alcohol intake above 50 standard drinks/week for at least 6 months resulted in >50% an attributable risk of cirrhosis, meaning that a significant proportion of HCV patients develops cirrhosis due to alcohol intake rather than HCV itself [94].

In addition to direct ethanol direct toxic effect, accelerated fibrosis progression in HCV patients has been linked to the synergistic interplay between ethanol and hepatitis C virus: indeed, alcohol abuse seems to reduce immune-mediated cellular response towards non-structural HCV proteins and to determine a Th2 polarization of immune responses [95]. Some other studies have suggested an ethanol-mediated promoting effect on viral replication, where HCV patients with alcohol abuse display higher viremia levels than HCV abstainers [96].

Whether daily or episodic alcohol intake is associated with the highest risk of liver damage has been also much debated, since binge drinking (defined as more than 50–60 g alcohol ingestion in a limited time period, usually 2 h) is becoming increasingly common: many studies conducted in alcohol-related liver disease suggest that daily alcohol intake confers the highest risk of developing liver damage [97–100], whereas other authors have reported an increased risk for binge drinking [101].

7.3.2.9 Steatosis, Obesity, and Insulin Resistance

Hepatic steatosis has been reported as an independent predictor of fibrosis progression by many studies: a French study prospectively evaluating 96 HCV patients

undergoing two liver biopsies at a mean interval of 48 months found 31% patients with evidence of fibrosis progression that was associated with male sex, worsening of histological activity, and worsening of steatosis. At multivariate analysis, worsening of steatosis was independently associated with liver fibrosis (OR 4.7 CI 1.3–10.8, $p = 0.0001$) [77]. These results have been confirmed by other cross-sectional studies, where steatosis correlated with fibrosis; visceral fat distribution and HCV genotype 3 infection were the other variables significantly associated with steatosis degree [102]. Steatosis at baseline liver biopsy was associated with worse clinical outcomes and progression of liver disease in 985 patients from the HALT-C study, where also insulin resistance defined by HOMA and weight changes predicted disease progression [103]. The independent association between steatosis and fibrosis has been also confirmed by a large meta-analysis conducted on 3068 patients with histologically confirmed HCV chronic infection [104]. Moreover, the pro-steatogenic role of HCV genotype 3 could partially explain data demonstrating a faster fibrosis progression in patients with HCV-3 chronic infection [72].

Steatosis is only a part of the complex interplay between HCV infection, insulin resistance, obesity, and metabolic syndrome, whose separate contribution in liver disease progression during chronic HCV infection is difficult to evaluate. The increased prevalence of type 2 diabetes mellitus (DM2) observed in HCV patient population has suggested that the virus itself may be also directly involved in DM2 pathogenic mechanisms [105]. Indeed, HCV may directly promote insulin resistance, the first step in the process leading to DM2 development, as the HCV core protein has been shown to inhibit insulin receptor-dependent signaling pathways [106]. Whenever virus-related or host-related in metabolic syndrome, insulin resistance and DM2 are key determinants of liver disease progression and risk factors for liver-related complications [107, 108].

Indeed, a recent nationwide cohort study from Taiwan enrolling 6251 patients has demonstrated that new onset of diabetes was associated with increased risk of cirrhosis and liver decompensation over a 10-year follow-up period. Patients with new-onset diabetes showed a significantly higher cumulative incidence of cirrhosis (RR = 1.53; 95% CI = 1.11–2.11; $p < 0.001$) and decompensated cirrhosis (RR = 2.01; 95% CI = 1.07–3.79, $p < 0.001$) compared to non-diabetic patients. After adjustment for age, gender, and comorbidities, diabetes was still an independent predictor for cirrhosis (HR = 2.505; 95% CI = 1.609–3.897; $p < 0.001$) and decompensation (HR = 3.560; 95% CI = 1.526–8.307; $p = 0.003$) [109].

It is important to underline that insulin resistance and diabetes have been identified by many studies as host factors negatively affecting chances of achieving HCV cure in the PegIFN + Rbv treatment era [110–113]: as a consequence, influence of diabetes on natural history of HCV infection in past studies could also result by a treatment bias where patients with DM2 were less likely to achieve HCV eradication. In addition, SVR to antiviral treatment has been shown to improve insulin resistance or partially prevent de novo insulin resistance development [114–117]. However, even after HCV cure, metabolic factors and especially presence of diabetes have been found as the strongest predictors of liver-related complications

long term, particularly hepatocellular carcinoma [118, 119], thus reinforcing the strong link between DM2 and worse prognosis in HCV infection.

7.4 HCV-Related Cirrhosis and Complications

From a clinical point of view, natural history of cirrhosis encompasses a large disease spectrum and is characterized by a continuum, where architectural alterations and loss of functioning parenchyma eventually result in liver dysfunction and development of cirrhosis complications, such as portal hypertension and ascites, variceal bleeding, hepatocellular carcinoma, and liver insufficiency. Irrespective from liver disease etiology, a systematic literature review by D'Amico and colleagues has proposed a classification of cirrhosis of in four clinical stages, where each stage is associated with different risk of clinical complications, survival rates, and prognosis. Stage 1 is characterized by compensated cirrhosis without esophageal varices or ascites, with excellent overall survival (90–95% at 5 years) and 1% mortality rate/year. Progression from stage 1 to other stages occurs in approximately 11% of patients/year, due to ascites onset or development of varices. Stage 2 is characterized by presence of varices without bleeding or ascites and displays a yearly 3.4% mortality rate. It is estimated that 6.6% and 4% of patients/year develop ascites or variceal bleeding, respectively, thus progressing to stage 3 and 4. Stage 3 is characterized by ascites with or without varices, with mortality increasing to 20%/year, whereas stage 4 is defined when gastrointestinal bleeding occurs with or without ascites; in this latter stage, 1-year mortality reaches 57%. Overall, stages 1 and 2 identify patients with compensated cirrhosis, while stages 3 and 4 refer to decompensated patients [120].

These figures remain true if considering HCV-related cirrhosis, as it is now established that compensated cirrhosis display good overall survival, whereas transition from compensated to decompensated cirrhosis and development of portal hypertension results in increased rates of liver-related complications and is associated with reduced survival and worse short-term prognosis.

A 17-year cohort study including 214 HCV patients with compensated cirrhosis reported 60 (28%) patients progressing from compensated (Child-Pugh A) to decompensated cirrhosis, 45 (21%) patients Child-Pugh class B, and 15 (7%) Child Pugh C, respectively, whereas 154 (72%) patients remained in compensated status. According to liver-related complications, HCC developed in 68 (32%), ascites in 50 (23%), jaundice in 36 (17%), upper gastrointestinal bleeding in 13 (6%), and encephalopathy in 2 (1%), with annual incidence rates of 3.9%, 2.9%, 2.0%, 0.7%, and 0.1%, respectively. HCC was the main cause of death (44%) and the first complication to occur in 58 (27%) patients, followed by ascites in 29 (14%), jaundice in 20 (9%), and upper gastrointestinal bleeding in 3 (1%), resulting in 4% annual mortality rate [121]. These figures were confirmed in another 10-year prospective study on 254 HCV cirrhotic patients, where 31% of patients had at least one complication at the end of study period. The most frequent complication was HCC that was also the first complication to develop, followed by ascites,

gastrointestinal bleeding, and encephalopathy [122]. Follow-up data from the large HALT-C study, originally designed to investigate the potential benefit of PegIFN maintenance treatment in advanced fibrosis patients failing to achieve the SVR, allowed evaluating clinical outcomes over 8-year follow-up in 1050 patients. Clinical events occurred in 7.5% cirrhotic patients/year: decompensation was the most common event followed by HCC development. After clinical decompensation, probability of a second liver-related event increased to 12.9%/year, and mortality resulted in 10%/year. Baseline platelet count, indicating more advanced disease at baseline, was a strong predictor of all clinical events. During the 8 years of follow-up, death or liver transplantation occurred in 31.5% of patients with cirrhosis [123].

Confirming the key role of portal hypertension as determinant of liver-related events and prognosis, the study by Bruno and colleagues in 194 HCV patients with a median follow-up of 14 years demonstrated that esophageal varices were associated with development of decompensation (HR = 2.09; 95% CI 1.33–3.30) and liver-related death (HR = 2.27; 95% CI 1.41–3.66). A MELD score > 10 predicted overall mortality (HR = 2.15; 95% CI 1.50–3.09), whereas overall survival of patients with MELD≤10 was 80% at 10 years. HCC occurrence increased the risk of decompensation fivefold (HR = 5.52; 95% CI 3.77–8.09). 131 patients developed decompensation (ascites, bleeding, hepatic encephalopathy), and 109 patients had HCC, resulting in 9 liver transplants and 158 deaths. Decompensation was the main determinant of mortality which respect to HCC [124]. These results were confirmed also by a larger prospective study on 402 patients with HCV-related compensated cirrhosis, where incidence of liver-related complications was higher in patients with cirrhosis stage 2 according to D'Amico classification, that is, patients with varices with respect to stage 1 (66% vs. 26%, $p < 0.001$). Indeed over a median of 176 weeks, 67 patients in stage 1 (22%) developed varices, and clinical decompensation occurred in 80 patients (20%). The 6-year cumulative overall mortality or liver transplantation was 15% and 45%, for stages 1 and 2, respectively ($p < 0.001$) [125].

Risk of HCC development in HCV-related liver disease has been estimated 3–5%/year in patients with cirrhosis [121, 122, 126, 127], while it is a rare event in chronic hepatitis: indeed advanced liver disease and older age as predictors of HCC development suggest that oncogenic risk increases in late phases of HCV natural history [127, 128]. As a result, according to epidemiological peculiarities in HCV infection spread across different countries, HCC is on the rise especially in Northern America and Europe, due to parenteral risk behaviors as main HCV risk factors, while is declining in several traditionally high-risk countries of the Mediterranean Europe, Japan, and Hong Kong, following effective measures of national health to prevent HCV diffusion through medical procedures [129, 130]. HCC onset is one of the most frequent complications during natural history of HCV-related cirrhosis and is often associated with clinical decompensation, resulting in increased liver-related mortality [121–124]: indeed, liver disease progression and decompensated cirrhosis are the main factors affecting outcome of HCC patients, as liver insufficiency prevents eligibility to radical options for HCC treatment [131].

In addition to age and cirrhosis, male gender and metabolic syndrome with insulin resistance and DM2 are other strong risk factors for HCC development, together with alcohol intake and HBV co-infection [85, 118, 128, 130, 132, 133]. Several attempts have been made in order to refine risk stratification in HCV patients through development of HCC risk scores, these models taking into account presence of known HCC risk factors [126, 134–136] (Table 7.1). However, these scores have been often developed in selected patient subsets, so that applicability to other settings is unclear and further validation in larger patient populations is needed.

Due to advancing molecular and genetic science, many studies have also tried to evaluate HCC molecular aberrations, in order to identify specific mutations or gene signatures associated with carcinogenesis. Indeed polymorphisms in the NBS1 gene, involved in DNA repair mechanisms, have been associated with HCC, where patients with homozygosis for the mutated NBS1 G allele carried a doubled risk of HCC development with respect to wild-type CC genotype [137]. A recent genome-wide association study, conducted in more than 400 patients, has also reported a strong association between the single nucleotide polymorphism rs17047200 in the tolloid-like 1 gene (TLL1) and HCC development: indeed carriage of the mutated allele T emerged as an independent risk factor for HCC, and levels of TLL1 messenger RNA have been found increased in HCV liver tissues in parallel with advancing fibrosis [138]. Molecular research has also focused on epigenetic alterations, where hypermethylation of promoters involved in key steps of cell proliferation have been described in HCC patients [139]. Finally, some studies have also pointed out importance of amino acid substitutions in HCV viral proteins, especially the core region, which has been associated with increased risk of carcinogenesis [140].

7.5 HCV Extrahepatic Manifestations

It is now recognized that HCV infection is a true systemic disease, as it is associated with many extrahepatic manifestations (EHMs) resulting in additional comorbidities and impaired quality of life in HCV patients [141, 142]. Strict relationship with HCV infection has been well established for some disorders, such as mixed cryoglobulinemia and hematologic malignancies like non-Hodgkin lymphoma, where HCV eradication represents a true etiologic treatment, thus confirming the direct pathogenic role of HCV replication [143]. The list of HCV-related disorders is currently on continuous update, since association with HCV infection has been also recognized for lichen planus, monoclonal gammopathies, as well as fibromyalgia and sicca syndrome [141]. In recent years, moreover, many studies have also demonstrated a strong association between HCV infection and insulin resistance, diabetes, cardiovascular risk, end-stage renal disease, and neurocognitive disorders [114, 117, 144]. This has been confirmed by large population studies demonstrating that HCV treatment is associated with improved extrahepatic outcomes, i.e., lower incidence of end-stage renal disease, acute coronary syndrome, and ischemic stroke [144, 145]. All in all, HCV eradication following antiviral treatment positively not

Table 7.1 HCC risk scores in HCV patients

Author	Patients	Fibrosis stage (Metavir)	Variables	Risk strata	HCC Predicted incidence	Time
Lok [126]	1.050	F3–F4	Age Race Platelets ALP Presence of esophageal varices Smoking	Low risk Medium risk High risk (Logarithmic model)	0.4% 4.2% 17.8%	5 years
El-Serag [134]	11.721	F4	Age ALT AFP Platelet count	Plot model	Predicted probability varying according to combination of variables	6 months
Chang [135]	1.252	F0–F4	Age Gender Platelet count AFP Presence of advanced fibrosis	Low risk (score $\leq$ 4) Medium risk (score 5–6) High risk (score $\geq$ 7)	2.68% 14.3% 25.7%	5 years
Poynard [136]	3.927	F0–F4	Fibrosis biomarker (FibroTest™) Transient Elastography (FibroScan®)	Fibrotest >0.74 Fibroscan >12.5 kPA	16.8% (Fibrotest) 12.7% (Fibroscan)	5–10 years

ALP alkaline phosphatase, *ALT* aminotransferases, *AFP* alpha-fetoprotein

only affects liver disease and liver-related complications but also results in reduced all-cause mortality in HCV-infected patients [146, 147].

For extensive discussion of HCV-related extrahepatic manifestations, see Volume II.

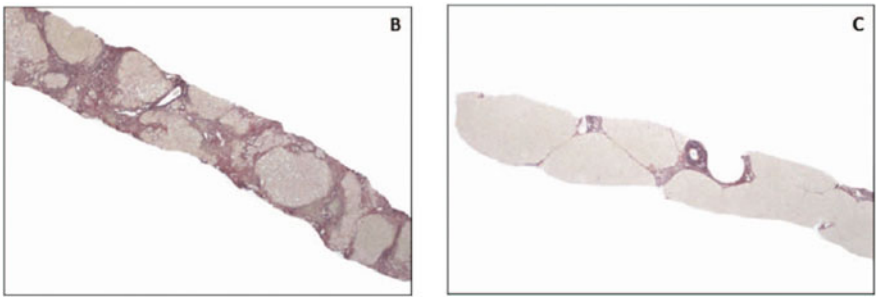
7.6 Natural History of Cirrhosis Following HCV Eradication

The long-standing dogma that cirrhosis should represent a no-return point has been challenged by many studies demonstrating that the achievement of the SVR following antiviral treatment, that is, HCV eradication, determines a clinical and histological improvement resulting in prevention of liver-related complications and increased survival.

Indeed several follow-up studies conducted in patients treated with PegIFN-based therapy have demonstrated liver fibrosis improvement and cirrhosis regression in 30–60% patients 3–5 years after the SVR [148–156] (Fig. 7.2).

Consistent evidence now exists that SVR has to be considered a real survival endpoint in HCV patients with advanced fibrosis, since it is associated with increased survival and reduction in all-cause mortality as well as liver-related mortality [64, 146, 157, 158].

In compensated cirrhosis, HCV cure improves portal hypertension: two pivotal papers have nicely demonstrated that the achievement of SVR prevents or delays de novo development of esophageal varices, although some patients are still at risk and persisting endoscopic surveillance is recommended in SVR patients with cirrhosis [159–161]. Along with portal hypertension prevention, SVR results in reduced risk of liver disease progression and clinical decompensation, eventually increasing survival of cirrhotic patients similarly to general population [157, 158, 162]. In decompensated cirrhosis, clinical trial and real-life data with new direct acting antivirals have consistently reported improvement of liver function and MELD



From D'Ambrosio et al, Hepatology 2012 [158]

B: Pre-treatment liver biopsy
C: Post-treatment liver biopsy (5 years after HCV eradication)

Fig. 7.2 Cirrhosis regression following achievement of HCV eradication

score in at least 30–50% of patients, although most currently available data refer to a 6–12 months observation period and longer follow-up is needed to prospectively confirm these evidences, as these patients still carry high risk of liver-related events in the short and long term [163–166].

Several works have provided strong evidence that achievement of SVR is associated with decreased incidence of HCC in patients with HCV-related cirrhosis; a meta-analysis evaluating 30 studies with 31.528 patients treated in the IFN-based era reported that SVR was associated with a reduced HCC risk in HCV patients with all stages of fibrosis (OR 0.24, 95% CI 0.18–0.31) [167]. More recently, a large cohort study from Veterans Affairs prospectively following 33.005 HCV patients treated with PegIFN ± Rbv from 1999 to 2009 reported a 0.33% annual HCC incidence in SVR patients vs. 1.32% in non SVR, confirming that SVR was associated with a significant reduction in HCC risk (HR: 0.49, 95% CI 0.46–0.53). However, annual HCC risk remained significantly higher among patients with cirrhosis (1.39%), meaning that these patients need to continue regular HCC surveillance overtime. Cirrhosis was identified as an independent predictor of HCC in the multivariate analysis together with age and diabetes [119]. A large revision of published clinical data about 1000 patients with advanced fibrosis/cirrhosis achieving the SVR has reported a 1% annual risk for HCC and 2% for disease progression; independent variables associated with residual HCC risk were age, diabetes, and lower platelet count, suggesting more advanced disease at baseline [168].

7.7 Conclusions

Natural history of HCV infection has been a challenging topic in last decades, where individual variability often prevented modeling of liver disease progression and many environmental factors acted as accelerators of fibrosis deposition. However, the scenario is rapidly changing, and availability of potent and effective antiviral treatments will modify natural history of HCV infection: indeed, increased efficacy together with worldwide scale up of treatment will reach the goal of HCV infection eradication in next decades. As a consequence, there will be a progressive shift from natural history of HCV infection to HCV-cured liver disease, where clinical outcomes and risk for liver-related complications are largely modified. However, even in this future scenario, cirrhotic patients will be still at risk of complications, and co-factors, especially metabolic syndrome, will play an important role as determinants of liver damage.

References

1. Seeff LB. Natural history of chronic hepatitis C. Hepatology. 2002;36:S35–46.
2. Gibb DM, Goodall RL, Dunn DT, et al. Mother-to-child transmission of hepatitis C virus: evidence for preventable peripartum transmission. Lancet. 2000;356:904–7.
3. Hoofnagle JH. Hepatitis C: the clinical spectrum of disease. Hepatology. 1997;26:15S–20S.

4. Orland JR, Wright TL, Cooper S. Acute hepatitis C. Hepatology. 2001;33:321–7.
5. Mosley JW, Operskalski EA, Tobler LH, et al. Viral and host factors in early hepatitis C virus infection. Hepatology. 2005;42:86–92.
6. Cox AL, Netski DM, Mosbruger T, et al. Prospective evaluation of community-acquired acute-phase hepatitis C virus infection. Clin Infect Dis. 2005;40:951–8.
7. Loomba R, Rivera MM, McBurney R, et al. The natural history of acute hepatitis C: clinical presentation, laboratory findings and treatment outcomes. Aliment Pharmacol Ther. 2011;33:559–65.
8. Deterding K, Wiegand J, Grüner N, et al. The German Hep-Net acute hepatitis C cohort: impact of viral and host factors on the initial presentation of acute hepatitis C virus infection. Z Gastroenterol. 2009;47:531–40.
9. Page K, Hahn JA, Evans J, et al. Acute hepatitis C virus infection in young adult injection drug users: a prospective study of incident infection, resolution, and reinfection. J Infect Dis. 2009;200:1216–26.
10. Rao HY, Sun DG, Jiang D, et al. IL28B genetic variants and gender are associated with spontaneous clearance of hepatitis C virus infection. J Viral Hepat. 2012;19:173–81.
11. Bakr I, Rekacewicz C, El Hosseiny M, et al. Higher clearance of hepatitis C virus infection in females compared with males. Gut. 2006;55:1183–7.
12. Micallef JM, Kaldor JM, Dore GJ. Spontaneous viral clearance following acute hepatitis C infection: a systematic review of longitudinal studies. J Viral Hepat. 2006;13:34–41.
13. Wang CC, Krantz E, Klarquist J, et al. Acute hepatitis C in a contemporary US cohort: modes of acquisition and factors influencing viral clearance. J Infect Dis. 2007;196:1474–82.
14. Ulsenheimer A, Lucas M, Seth NP, et al. Transient immunological control during acute hepatitis C virus infection: ex vivo analysis of helper T-cell responses. J Viral Hepat. 2006;13:708–14.
15. Kaplan DE, Sugimoto K, Newton K, et al. Discordant role of CD4 T-cell response relative to neutralizing antibody and CD8 T-cell responses in acute hepatitis C. Gastroenterology. 2007;132:654–66.
16. Gerlach JT, Diepolder HM, Jung MC, et al. Recurrence of hepatitis C virus after loss of virus-specific CD4(+) T-cell response in acute hepatitis C. Gastroenterology. 1999;117:933–41.
17. Li K, Foy E, Ferreon JC, et al. Immune evasion by hepatitis C virus NS3/4A protease-mediated cleavage of the Toll-like receptor 3 adaptor protein TRIF. Proc Natl Acad Sci U S A. 2005;102:2992–7.
18. Cabrera R, Tu Z, Xu Y, et al. An immunomodulatory role for CD4(+)CD25(+) regulatory T lymphocytes in hepatitis C virus infection. Hepatology. 2004;40:1062–71.
19. Bowen DG, Walker CM. Adaptive immune responses in acute and chronic hepatitis C virus infection. Nature. 2005;436:946–52.
20. Alter G, Jost S, Rihn S, et al. Reduced frequencies of NKp30+NKp46+, CD161+, and NKG2D+ NK cells in acute HCV infection may predict viral clearance. J Hepatol. 2011;55:278–88.
21. Varchetta S, Mele D, Mantovani S, et al. Impaired intrahepatic natural killer cell cytotoxic function in chronic hepatitis C virus infection. Hepatology. 2012;56:841–9.
22. Ge D, Fellay J, Thompson AJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature. 2009;461:399–401.
23. Thomas DL, Thio CL, Martin MP, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature. 2009;461:798–801.
24. Tillmann HL, Thompson AJ, Patel K, et al. A polymorphism near IL28B is associated with spontaneous clearance of acute hepatitis C virus and jaundice. Gastroenterology. 2010;139:1586–92.
25. Grebely J, Dore GJ, Kim AY, et al. Genetics of spontaneous clearance of hepatitis C virus infection: a complex topic with much to learn. Hepatology. 2014;60:2127–8.
26. Rousselet MC, Michalak S, Dupré F, et al. Sources of variability in histological scoring of chronic viral hepatitis. Hepatology. 2005;41:257–64.

27. Ghany MG, Kleiner DE, Alter H, et al. Progression of fibrosis in chronic hepatitis C. Gastroenterology. 2003;124:97–104.
28. Ryder SD, Irving WL, Jones DA, et al. Progression of hepatic fibrosis in patients with hepatitis C: a prospective repeat liver biopsy study. Gut. 2004;53:451–5.
29. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. Lancet. 1997;349:825–32.
30. Tong MJ, El-Farra NS, Reikes AR, et al. Clinical outcomes after transfusion-associated hepatitis C. N Engl J Med. 1995;332:1463–6.
31. Yano M, Kumada H, Kage M, et al. The long-term pathological evolution of chronic hepatitis C. Hepatology. 1996;23:1334–40.
32. Missiha SB, Ostrowski M, Heathcote EJ. Disease progression in chronic hepatitis C: modifiable and nonmodifiable factors. Gastroenterology. 2008;134:1699–714.
33. Wiese M, Grüngreiff K, Güthoff W, et al. Outcome in a hepatitis C (genotype 1b) single source outbreak in Germany—a 25-year multicenter study. J Hepatol. 2005;43:590–8.
34. Kenny-Walsh E. Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. Irish Hepatology Research Group. N Engl J Med. 1999;340:1228–33.
35. Wiese M, Berr F, Lafrenz M, et al. Low frequency of cirrhosis in a hepatitis C (genotype 1b) single-source outbreak in Germany: a 20-year multicenter study. Hepatology. 2000;32:91–6.
36. Thomas DL, Astemborski J, Rai RM, et al. The natural history of hepatitis C virus infection: host, viral, and environmental factors. JAMA. 2000;284:450–6.
37. Thein HH, Yi Q, Dore GJ, et al. Estimation of stage-specific fibrosis progression rates in chronic hepatitis C virus infection: a meta-analysis and meta-regression. Hepatology. 2008;48:418–31.
38. Vogt M, Lang T, Frösner G, et al. Prevalence and clinical outcome of hepatitis C infection in children who underwent cardiac surgery before the implementation of blood-donor screening. N Engl J Med. 1999;341:866–70.
39. Svirtlih N, Jevtovic D, Simonovic J, et al. Older age at the time of liver biopsy is the important risk factor for advanced fibrosis in patients with chronic hepatitis C. Hepato-Gastroenterology. 2007;54:2324–7.
40. Zarski JP, Mc Hutchison J, Bronowicki JP, et al. Rate of natural disease progression in patients with chronic hepatitis C. J Hepatol. 2003;38:307–14.
41. Wiese M, Fischer J, Löbermann M, et al. Evaluation of liver disease progression in the German hepatitis C virus (1b)-contaminated anti-D cohort at 35 years after infection. Hepatology. 2014;59:49–57.
42. Shimizu I, Mizobuchi Y, Yasuda M, et al. Inhibitory effect of oestradiol on activation of rat hepatic stellate cells in vivo and in vitro. Gut. 1999;44:127–36.
43. Yasuda M, Shimizu I, Shiba M, et al. Suppressive effects of estradiol on dimethylnitrosamine-induced fibrosis of the liver in rats. Hepatology. 1999;29:719–27.
44. Villa E, Vukotic R, Cammà C, et al. Reproductive status is associated with the severity of fibrosis in women with hepatitis C. PLoS One. 2012;7:e44624.
45. Di Martino V, Lebray P, Myers RP, et al. Progression of liver fibrosis in women infected with hepatitis C: long-term benefit of estrogen exposure. Hepatology. 2004;40:1426–33.
46. Sterling RK, Stravitz RT, Luketic VA, et al. A comparison of the spectrum of chronic hepatitis C virus between Caucasians and African Americans. Clin Gastroenterol Hepatol. 2004;2:469–73.
47. Wiley TE, Brown J, Chan J. Hepatitis C infection in African Americans: its natural history and histological progression. Am J Gastroenterol. 2002;97:700–6.
48. Nguyen GC, Segev DL, Thuluvath PJ. Racial disparities in the management of hospitalized patients with cirrhosis and complications of portal hypertension: a national study. Hepatology. 2007;45:1282–9.
49. Kallwitz ER, Layden-Almer J, Dhamija M, et al. Ethnicity and body mass index are associated with hepatitis C presentation and progression. Clin Gastroenterol Hepatol. 2010;8:72–8.

50. Lawson A, Trent Hepatitis C Study Group. A comparison of the natural history and outcome of treatment for Asian and non-Asian hepatitis C-infected patients. J Viral Hepat. 2011;18: e270–7.
51. Tillmann HL, Chen DF, Trautwein C, et al. Low frequency of HLA-DRB1*11 in hepatitis C virus induced end stage liver disease. Gut. 2001;48:714–8.
52. Thursz M, Yallop R, Goldin R, et al. Influence of MHC class II genotype on outcome of infection with hepatitis C virus. The HENCORE group. Hepatitis C European Network for Cooperative Research. Lancet. 1999;354:2119–24.
53. Asselah T, Bièche I, Laurendeau I, et al. Liver gene expression signature of mild fibrosis in patients with chronic hepatitis C. Gastroenterology. 2005;129:2064–75.
54. Patin E, Kutalik Z, Guergnon J, et al. Genome-wide association study identifies variants associated with progression of liver fibrosis from HCV infection. Gastroenterology. 2012;143:1244–52.
55. Rüeger S, Bochud PY, Dufour JF, et al. Impact of common risk factors of fibrosis progression in chronic hepatitis C. Gut. 2015;64:1605–15.
56. Fabris C, Falleti E, Cussigh A, et al. IL-28B rs12979860 C/T allele distribution in patients with liver cirrhosis: role in the course of chronic viral hepatitis and the development of HCC. J Hepatol. 2011;54:716–22.
57. Marabita F, Aghemo A, De Nicola S, et al. Genetic variation in the interleukin-28B gene is not associated with fibrosis progression in patients with chronic hepatitis C and known date of infection. Hepatology. 2011;54:1127–34.
58. Valenti L, Rumi M, Galmozzi E, et al. Patatin-like phospholipase domain-containing 3 I148M polymorphism, steatosis, and liver damage in chronic hepatitis C. Hepatology. 2011;53:791–9.
59. Trépo E, Pradat P, Potthoff A, et al. Impact of patatin-like phospholipase-3 (rs738409 C>G) polymorphism on fibrosis progression and steatosis in chronic hepatitis C. Hepatology. 2011;54:60–9.
60. Hatzakis A, Katsoulidou A, Kaklamani E, et al. Hepatitis C virus 1b is the dominant genotype in HCV-related carcinogenesis: a case-control study. Int J Cancer. 1996;68:51–3.
61. Martinot-Peignoux M, Roudot-Thoraval F. Mendel I,et al. Hepatitis C virus genotypes in France: relationship with epidemiology, pathogenicity and response to interferon therapy. The GEMHEP. J Viral Hepat. 1999;6:435–43.
62. Silini E, Bottelli R, Asti M, et al. Hepatitis C virus genotypes and risk of hepatocellular carcinoma in cirrhosis: a case-control study. Gastroenterology. 1996;111:199–205.
63. Fattovich G, Ribero ML, Pantalena M, et al. Hepatitis C virus genotypes: distribution and clinical significance in patients with cirrhosis type C seen at tertiary referral centres in Europe. J Viral Hepat. 2001;8:206–16.
64. Bruno S, Crosignani A, Maisonneuve P, et al. Hepatitis C virus genotype 1b as a major risk factor associated with hepatocellular carcinoma in patients with cirrhosis: a seventeen-year prospective cohort study. Hepatology. 2007;46:1350–6.
65. Benvegnù L, Pontisso P, Cavalletto D, et al. Lack of correlation between hepatitis C virus genotypes and clinical course of hepatitis C virus-related cirrhosis. Hepatology. 1997;25:211–5.
66. Poynard T, Ratziu V, Charlotte F, et al. Rates and risk factors of liver fibrosis progression in patients with chronic hepatitis C. J Hepatol. 2001;34:730–9.
67. Mihm S, Fayyazi A, Hartmann H, et al. Analysis of histopathological manifestations of chronic hepatitis C virus infection with respect to virus genotype. Hepatology. 1997;25:735–9.
68. Rumi MG, De Filippi F, Donato MF, et al. Progressive hepatic fibrosis in healthy carriers of hepatitis C virus with a transaminase breakthrough. J Viral Hepat. 2002;9:71–4.
69. Rumi MG, De Filippi F, La Vecchia C, et al. Hepatitis C reactivation in patients with chronic infection with genotypes 1b and 2c: a retrospective cohort study of 206 untreated patients. Gut. 2005;54:402–6.

70. Rubbia-Brandt L, Fabris P, Paganin S, et al. Steatosis affects chronic hepatitis C progression in a genotype specific way. Gut. 2004;53:406–12.
71. Moriya K, Yotsuyanagi H, Shintani Y, et al. Hepatitis C virus core protein induces hepatic steatosis in transgenic mice. J Gen Virol. 1997;78:1527–31.
72. Bochud PY, Cai T, Overbeck K, et al. Genotype 3 is associated with accelerated fibrosis progression in chronic hepatitis C. J Hepatol. 2009;51:655–66.
73. Probst A, Dang T, Bochud M, et al. Role of hepatitis C virus genotype 3 in liver fibrosis progression—a systematic review and meta-analysis. J Viral Hepat. 2011;18:745–59.
74. Hui CK, Belaye T, Montegrande K, et al. A comparison in the progression of liver fibrosis in chronic hepatitis C between persistently normal and elevated transaminase. J Hepatol. 2003;38:511–7.
75. Mathurin P, Moussalli J, Cadranel JF, et al. Slow progression rate of fibrosis in hepatitis C virus patients with persistently normal alanine transaminase activity. Hepatology. 1998;27:868–72.
76. Puoti C, Castellacci R, Montagnese F, et al. Histological and virological features and follow-up of hepatitis C virus carriers with normal aminotransferase levels: the Italian prospective study of the asymptomatic C carriers (ISACC). J Hepatol. 2002;37:117–23.
77. Castéra L, Hézode C, Roudot-Thoraval F, et al. Worsening of steatosis is an independent factor of fibrosis progression in untreated patients with chronic hepatitis C and paired liver biopsies. Gut. 2003;52:288–92.
78. Shiffman ML, Diago M, Tran A, et al. Chronic hepatitis C in patients with persistently normal alanine transaminase levels. Clin Gastroenterol Hepatol. 2006;4:645–52.
79. European Association for the Study of the Liver. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatol. 2017;67(2):370–98.
80. Aghemo A, Colombo M. Treatment of patients with dual hepatitis B and C: a step in the right direction. Gut. 2014;63:380–1.
81. Fattovich G, Tagger A, Brollo L, et al. Hepatitis C virus infection in chronic hepatitis B virus carriers. J Infect Dis. 1991;163:400–2.
82. Crespo J, Lozano JL, de la Cruz F, et al. Prevalence and significance of hepatitis C viremia in chronic active hepatitis B. Am J Gastroenterol. 1994;89:1147–51.
83. Zarski JP, Bohn B, Bastie A, et al. Characteristics of patients with dual infection by hepatitis B and C viruses. J Hepatol. 1998;28:27–33.
84. Viganò M, Aghemo A, Iavarone M, et al. The course of inactive hepatitis B in hepatitis-C-coinfected patients treated with interferon and ribavirin. Antivir Ther. 2009;14:789–96.
85. Caccamo G, Saffioti F, Raimondo G. Hepatitis B virus and hepatitis C virus dual infection. World J Gastroenterol. 2014;20:14559–67.
86. Cho LY, Yang JJ, Ko KP, et al. Coinfection of hepatitis B and C viruses and risk of hepatocellular carcinoma: systematic review and meta-analysis. Int J Cancer. 2011;128:176–84.
87. Thein HH, Yi Q, Dore GJ, et al. Natural history of hepatitis C virus infection in HIV-infected individuals and the impact of HIV in the era of highly active antiretroviral therapy: a meta-analysis. AIDS. 2008;22:1979–91.
88. Martin-Carbonero L, de Ledinghen V, Moreno A, et al. Liver fibrosis in patients with chronic hepatitis C and persistently normal liver enzymes: influence of HIV infection. J Viral Hepat. 2009;16:790–58.
89. Graham CS, Baden LR, Yu E, et al. Influence of human immunodeficiency virus infection on the course of hepatitis C virus infection: a meta-analysis. Clin Infect Dis. 2001;33:562–9.
90. European Association for Study of Liver. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. J Hepatol. 2017;66:153–94.
91. Turner C. How much alcohol is in a ‘standard drink’? An analysis of 125 studies. Br J Addict. 1990;85:1171–5.
92. O’Shea RS, Dasarathy S, McCullough AJ. Alcoholic liver disease. Hepatology. 2010;51:307–28.

93. Hézode C, Lonjon I, Roudot-Thoraval F, et al. Impact of moderate alcohol consumption on histological activity and fibrosis in patients with chronic hepatitis C, and specific influence of steatosis: a prospective study. Aliment Pharmacol Ther. 2003;17:1031–7.
94. Innes HA, Hutchinson SJ, Barclay S, et al. Quantifying the fraction of cirrhosis attributable to alcohol among chronic hepatitis C virus patients: implications for treatment cost-effectiveness. Hepatology. 2013;57:451–601.
95. Aloman C, Gehring S, Wintermeyer P, et al. Chronic ethanol consumption impairs cellular immune responses against HCV NS5 protein due to dendritic cell dysfunction. Gastroenterology. 2007;132:698–708.
96. Zhang T, Li Y, Lai JP, et al. Alcohol potentiates hepatitis C virus replicon expression. Hepatology. 2003;38:57–65.
97. Dawson DA, Li TK, Grant BF. A prospective study of risk drinking: at risk for what? Drug Alcohol Depend. 2008;95:62–72.
98. Stokkeland K, Hilm G, Spak F, et al. Different drinking patterns for women and men with alcohol dependence with and without alcoholic cirrhosis. Alcohol Alcohol. 2008;43:39–45.
99. Hatton J, Burton A, Nash H, et al. Drinking patterns, dependency and life-time drinking history in alcohol-related liver disease. Addiction. 2009;104:587–92.
100. Askgaard G, Grønbæk M, Kjær MS, et al. Alcohol drinking pattern and risk of alcoholic liver cirrhosis: a prospective cohort study. J Hepatol. 2015;62:1061–7.
101. Parrish KM, Dufour MC, Stinson FS, et al. Average daily alcohol consumption during adult life among decedents with and without cirrhosis: the 1986 National Mortality Followback Survey. J Stud Alcohol. 1993;54:450–6.
102. Adinolfi LE, Gambardella M, Andreana A, et al. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. Hepatology. 2001;33:1358–64.
103. Everhart JE, Lok AS, Kim HY, et al. Weight-related effects on disease progression in the hepatitis C antiviral long-term treatment against cirrhosis trial. Gastroenterology. 2009;137:549–57.
104. Leandro G, Mangia A, Hui J, et al. Relationship between steatosis, inflammation, and fibrosis in chronic hepatitis C: a meta-analysis of individual patient data. Gastroenterology. 2006;130:1636–42.
105. White DL, Ratziu V, El-Serag HB. Hepatitis C infection and risk of diabetes: a systematic review and meta-analysis. J Hepatol. 2008;49:831–44.
106. Moucari R, Asselah T, Cazals-Hatem D, et al. Insulin resistance in chronic hepatitis C: association with genotypes 1 and 4, serum HCV RNA level, and liver fibrosis. Gastroenterology. 2008;134:416–23.
107. Fartoux L, Poujol-Robert A, Guéchot J, et al. Insulin resistance is a cause of steatosis and fibrosis progression in chronic hepatitis C. Gut. 2005;54:1003–8.
108. Hui JM, Sud A, Farrell GC, et al. Insulin resistance is associated with chronic hepatitis C virus infection and fibrosis progression. Gastroenterology. 2003;125:1695–704.
109. Huang YW, Yang SS, Fu SC, et al. Increased risk of cirrhosis and its decompensation in chronic hepatitis C patients with new-onset diabetes: a nationwide cohort study. Hepatology. 2014;60:807–14.
110. Romero-Gómez M, Del Mar Viloria M, Andrade RJ, et al. Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in chronic hepatitis C patients. Gastroenterology. 2005;128:636–41.
111. Deltenre P, Louvet A, Lemoine M, et al. Impact of insulin resistance on sustained response in HCV patients treated with pegylated interferon and ribavirin: a meta-analysis. J Hepatol. 2011;55:1187–94.
112. Brandman D, Bacchetti P, Ayala CE, et al. Impact of insulin resistance on HCV treatment response and impact of HCV treatment on insulin sensitivity using direct measurements of insulin action. Diabetes Care. 2012;35:1090–4.

113. Eslam M, Aparcero R, Kawaguchi T, et al. Meta-analysis: insulin resistance and sustained virological response in hepatitis C. Aliment Pharmacol Ther. 2011;34:297–305.
114. Arase Y, Suzuki F, Suzuki Y, et al. Sustained virological response reduces incidence of onset of type 2 diabetes in chronic hepatitis C. Hepatology. 2009;49:739–44.
115. Romero-Gómez M, Fernández-Rodríguez CM, Andrade RJ, et al. Effect of sustained virological response to treatment on the incidence of abnormal glucose values in chronic hepatitis C. J Hepatol. 2008;48:721–7.
116. Thompson AJ, Patel K, Chuang WL, et al. Viral clearance is associated with improved insulin resistance in genotype 1 chronic hepatitis C but not genotype 2/3. Gut. 2012;61:128–34.
117. Aghemo A, Prati GM, Rumi MG, et al. Sustained virological response prevents the development of insulin resistance in patients with chronic hepatitis C. Hepatology. 2012;56:1681–7.
118. Arase Y, Kobayashi M, Suzuki F, et al. Effect of type 2 diabetes on risk for malignancies includes hepatocellular carcinoma in chronic hepatitis C. Hepatology. 2013;57:964–73.
119. El-Serag HB, Kanwal F, Richardson P, et al. Risk of hepatocellular carcinoma after sustained virological response in Veterans with hepatitis C virus infection. Hepatology. 2016;64:130–7.
120. D'Amico G, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. J Hepatol. 2006;44:217–31.
121. Sangiovanni A, Prati GM, Fasani P, et al. The natural history of compensated cirrhosis due to hepatitis C virus: A 17-year cohort study of 214 patients. Hepatology. 2006;43:1303–10.
122. Benvegnù L, Gios M, Boccato S, et al. Natural history of compensated viral cirrhosis: a prospective study on the incidence and hierarchy of major complications. Gut. 2004;53:744–9.
123. Dienstag JL, Ghany MG, Morgan TR, et al. A prospective study of the rate of progression in compensated, histologically advanced chronic hepatitis C. Hepatology. 2011;54:396–405.
124. Bruno S, Zuin M, Crosignani A, et al. Predicting mortality risk in patients with compensated HCV-induced cirrhosis: a long-term prospective study. Am J Gastroenterol. 2009;104:1147–58.
125. Gomez EV, Rodriguez YS, Bertot LC, et al. The natural history of compensated HCV-related cirrhosis: a prospective long-term study. J Hepatol. 2013;58:434–44.
126. Lok AS, Seeff LB, Morgan TR, et al. Incidence of hepatocellular carcinoma and associated risk factors in hepatitis C-related advanced liver disease. Gastroenterology. 2009;136:138–48.
127. Degos F, Christidis C, Ganne-Carrie N, et al. Hepatitis C virus related cirrhosis: time to occurrence of hepatocellular carcinoma and death. Gut. 2000;47:131–6.
128. McCombs J, Matsuda T, Tonnu-Mihara I, et al. The risk of long-term morbidity and mortality in patients with chronic hepatitis C: results from an analysis of data from a Department of Veterans Affairs Clinical Registry. JAMA Intern Med. 2014;174:204–12.
129. Hatzakis A, Chulanov V, Gadano AC, et al. The present and future disease burden of hepatitis C virus (HCV) infections with today's treatment paradigm. J Viral Hepat. 2015;22:S26–45.
130. Petrick JL, Kelly SP, Altekruse SF, et al. Future of hepatocellular carcinoma incidence in the United States forecast through 2030. J Clin Oncol. 2016;34:1787–94.
131. European Association for the Study of the Liver European Organisation for Research and Treatment of Cancer. EASL–EORTC Clinical Practice Guidelines: management of hepatocellular carcinoma. J Hepatol. 2015;56:908–46.
132. Veldt BJ, Chen W, Heathcote EJ, et al. Increased risk of hepatocellular carcinoma among patients with hepatitis C cirrhosis and diabetes mellitus. Hepatology. 2008;47:1856–62.
133. Vandenbulcke H, Moreno C, Colle I, et al. Alcohol intake increases the risk of HCC in hepatitis C virus-related compensated cirrhosis: A prospective study. J Hepatol. 2016;65:543–51.
134. El-Serag HB, Kanwal F, Davila JA, et al. A new laboratory-based algorithm to predict development of hepatocellular carcinoma in patients with hepatitis C and cirrhosis. Gastroenterology. 2014;146:1249–55.
135. Chang KC, Wu YY, Hung CH, et al. Clinical-guide risk prediction of hepatocellular carcinoma development in chronic hepatitis C patients after interferon-based therapy. Br J Cancer. 2013;109:2481–8.

136. Poynard T, Vergniol J, Ngo Y, et al. Staging chronic hepatitis C in seven categories using fibrosis biomarker (FibroTest™) and transient elastography (FibroScan®). J Hepatol. 2014;60:706–14.
137. Huang MD, Chen XF, Xu G, et al. Genetic variation in the NBS1 gene is associated with hepatic cancer risk in a Chinese population. DNA Cell Biol. 2012;31:678–82.
138. Matsuura K, Sawai H, Ikeo K, et al. Genome-wide association study identifies TLL1 variant associated with development of hepatocellular carcinoma after eradication of hepatitis C virus infection. Gastroenterology. 2017;152:1383–94.
139. Hayashi T, Tamori A, Nishikawa M, et al. Differences in molecular alterations of hepatocellular carcinoma between patients with a sustained virological response and those with hepatitis C virus infection. Liver Int. 2009;29:126–32.
140. Akuta N, Suzuki F, Hirakawa M, et al. Amino acid substitutions in hepatitis C virus core region predict hepatocarcinogenesis following eradication of HCV RNA by antiviral therapy. J Med Virol. 2011;83:1016–22.
141. Zignego AL, Ramos-Casals M, Ferri C, et al. International therapeutic guidelines for patients with HCV-related extrahepatic disorders. A multidisciplinary expert statement. Autoimmun Rev. 2017;16:523–41.
142. Younossi Z, Park H, Henry L, et al. Extrahepatic manifestations of hepatitis C: a meta-analysis of prevalence, quality of life, and economic burden. Gastroenterology. 2016;150:1599–608.
143. Kawamura Y, Ikeda K, Arase Y, et al. Viral elimination reduces incidence of malignant lymphoma in patients with hepatitis C. Am J Med. 2007;120:1034–41.
144. Hsu YC, Ho HJ, Huang YT, et al. Association between antiviral treatment and extrahepatic outcomes in patients with hepatitis C virus infection. Gut. 2015;64:495–503.
145. Hsu YC, Lin JT, Ho HJ, et al. Antiviral treatment for hepatitis C virus infection is associated with improved renal and cardiovascular outcomes in diabetic patients. Hepatology. 2014;59:1293–302.
146. van der Meer AJ, Veldt BJ, Feld JJ, et al. Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis. JAMA. 2012;308:2584–93.
147. Tada T, Kumada T, Toyoda H, et al. Viral eradication reduces all-cause mortality in patients with chronic hepatitis C virus infection: a propensity score analysis. Liver Int. 2016;36:817–26.
148. Reichard O, Glaumann H, Frydén A, et al. Long-term follow-up of chronic hepatitis C patients with sustained virological response to alpha-interferon. J Hepatol. 1999;30:783–7.
149. Shiratori Y, Imazeki F, Moriyama M, et al. Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy. Ann Intern Med. 2000;132:517–24.
150. Poynard T, McHutchison J, Manns M, et al. Impact of pegylated interferon alfa-2b and ribavirin on liver fibrosis in patients with chronic hepatitis C. Gastroenterology. 2002;122:1303–13.
151. Arif A, Levine RA, Sanderson SO, et al. Regression of fibrosis in chronic hepatitis C after therapy with interferon and ribavirin. Dig Dis Sci. 2003;48:1425–30.
152. Pol S, Carnot F, Nalpas B, et al. Reversibility of hepatitis C virus-related cirrhosis. Hum Pathol. 2004;35:107–12.
153. Mallet V, Gilgenkrantz H, Serpaggi J, et al. Brief communication: the relationship of regression of cirrhosis to outcome in chronic hepatitis C. Ann Intern Med. 2008;149:399–403.
154. Everson GT, Balart L, Lee SS, et al. Histological benefits of virological response to peginterferon alfa-2a monotherapy in patients with hepatitis C and advanced fibrosis or compensated cirrhosis. Aliment Pharmacol Ther. 2008;27:542–51.
155. George SL, Bacon BR, Brunt EM, et al. Clinical, virologic, histologic, and biochemical outcomes after successful HCV therapy: a 5-year follow-up of 150 patients. Hepatology. 2009;49:729–38.

156. D'Ambrosio R, Aghemo A, Rumi MG, et al. A morphometric and immunohistochemical study to assess the benefit of a sustained virological response in hepatitis C virus patients with cirrhosis. Hepatology. 2012;56:532–43.
157. Bruno S, Di Marco V, Iavarone M, et al. Improved survival of patients with hepatocellular carcinoma and compensated hepatitis C virus-related cirrhosis who attained sustained virological response. Liver Int. 2017;37(10):1526–34. https://doi.org/10.1111/liv.13452.
158. Petta S, Di Marco V, Bruno S, et al. Impact of virus eradication in patients with compensated hepatitis C virus-related cirrhosis: competing risks and multistate model. Liver Int. 2016;36:1765–73.
159. Bruno S, Crosignani A, Facciotto C, et al. Sustained virologic response prevents the development of esophageal varices in compensated, Child-Pugh class A hepatitis C virus-induced cirrhosis. A 12-year prospective follow-up study. Hepatology. 2010;51:2069–76.
160. D'Ambrosio R, Aghemo A, Rumi MG, et al. The course of esophageal varices in patients with hepatitis C cirrhosis responding to interferon/ribavirin therapy. Antivir Ther. 2011;16:677–84.
161. de Franchis R, Baveno VI. Expanding consensus in portal hypertension: Report of the Baveno VI Consensus Workshop: Stratifying risk and individualizing care for portal hypertension. J Hepatol. 2015;63:743–52.
162. Bruno S, Di Marco V, Iavarone M, et al. Survival of patients with HCV cirrhosis and sustained virologic response is similar to the general population. J Hepatol. 2016;64:1217–23.
163. Charlton M, Everson GT, Flamm SL, et al. Ledipasvir and sofosbuvir plus ribavirin for treatment of HCV infection in patients with advanced liver disease. Gastroenterology. 2015;149:649–59.
164. Manns M, Samuel D, Gane EJ, et al. Ledipasvir and sofosbuvir plus ribavirin in patients with genotype 1 or 4 hepatitis C virus infection and advanced liver disease: a multicentre, open-label, randomised, phase 2 trial. Lancet Infect Dis. 2016;16:685–97.
165. Fernández Carrillo C, Lens S, Llop E, et al. Treatment of hepatitis C virus infection in patients with cirrhosis and predictive value of model for end-stage liver disease: Analysis of data from the Hepa-C registry. Hepatology. 2017;65:1810–22.
166. Foster GR, Irving WL, Cheung MC, et al. Impact of direct acting antiviral therapy in patients with chronic hepatitis C and decompensated cirrhosis. J Hepatol. 2016;64:1224–31.
167. Morgan RL, Baack B, Smith BD, et al. Eradication of hepatitis C virus infection and the development of hepatocellular carcinoma: a meta-analysis of observational studies. Ann Intern Med. 2013;158:329–37.
168. van der Meer AJ, Feld JJ, et al. Risk of cirrhosis-related complications in patients with advanced fibrosis following hepatitis C virus eradication. J Hepatol. 2017;66:485–93.

Epidemiology: Modeling of Natural History

8

Vana Sypsa

8.1 Introduction

The natural history of a disease refers to the course of the disease over time in the absence of treatment. As it has been detailed in previous chapters, infection with HCV can progress to liver fibrosis and may lead to the development of cirrhosis, HCC, liver failure, and death. The collection of these stages constitutes the natural history of the disease. A large number of retrospective and prospective studies on chronic hepatitis C (CHC) patients have allowed to gain insight into the various stages of the disease as well as the time course of disease progression. The latter is important as it relates to the rate at which patients progress through disease stages.

Models have been widely employed, in particular in infectious diseases, to provide a simplified representation of complex phenomena and to obtain useful estimates. A model describing the natural history of a disease consists of disease stages as well as allowed transitions between them. Once a proper model structure is established, then patients are assumed to transition between disease stages based on a set of transition probabilities. Thus, models consist of three important ingredients: disease stages, allowed transitions between stages, and corresponding stage-specific transition probabilities. In the case of CHC, models aim to mimic the course of the disease from infection to the occurrence of serious sequelae (cirrhosis, HCC, death) as in the example of Fig. 8.1.

This representation can be very convenient as the slow progression of CHC makes it difficult to obtain data covering the whole course of the disease. Employing a series of stages, with appropriate transitions between them, allows to make use of epidemiological studies providing estimates on the rate of progression between stages, e.g., on the annual probability of developing HCC among cirrhotic patients

V. Sypsa (✉)
Department of Hygiene, Epidemiology and Medical Statistics, Medical School, National and Kapodistrian University of Athens, Athens, Greece
e-mail: vsipsa@med.uoa.gr

A. Hatzakis (ed.), *Hepatitis C: Epidemiology, Prevention and Elimination*,
https://doi.org/10.1007/978-3-030-64649-3_8

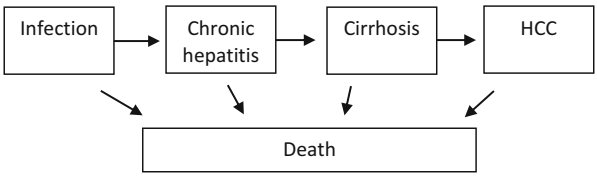


Fig. 8.1 A simple model describing the natural history of chronic hepatitis C. The squares denote the disease stages of the model (five disease stages: infection, chronic infection, cirrhosis, hepatocellular carcinoma, and death). The arrows denote the allowed transitions between disease stages

or of death among HCC patients. If there are reliable estimates of these transition probabilities, then it is possible to employ the model and synthesize information to obtain estimates on the risk of developing cirrhosis, of HCC, and of liver-related mortality since the onset of infection. If estimates of transition probabilities are available according to patients' characteristics (e.g., age and/or gender), then more refined estimates of the time to develop cirrhosis and other sequelae can be obtained.

Models can be also used to estimate stage-specific transition probabilities based on the fit of the model to a set of observed data. For example, Deuffic et al. [1] estimated the annual probability of progression from chronic hepatitis to cirrhosis that provided the closest fit between the observed data on HCV-related HCC deaths in France and the corresponding model predictions. It is possible to estimate these probabilities according to important patient characteristics, such as age and gender.

Apart from the usefulness of models in gaining a better understanding of the natural history of CHC, they are also extensively used to investigate public health policy questions. Thus, it is possible to use them to estimate the course of the disease for a given population, for example, HCV-infected persons in a country, and project the future morbidity and mortality burden of CHC [1–8]. Another interesting application is to incorporate the impact of prevention and/or treatment in the models and use them to assess the impact of alternative prevention and treatment strategies in reducing the future burden of CHC and to evaluate the associate costs [9–21]. The latter however is out of the scope of this chapter.

To summarize, models of natural history of CHC can be used to estimate parameters related to the natural history of HCV as well as to project the disease burden anticipated in the following years. They can be also used to compare the impact of alternative strategies on the future burden. They are important at an individual level, as they produce individualized information on risk of disease progression, and also at population level as they allow to estimate liver disease burden in a given population [22].

This chapter reviews the natural history models that have been used for CHC. First, it discusses the various challenges in modeling the long, asymptomatic period before the occurrence of serious sequelae. Then, it reviews the approaches and methods that have been used to estimate the rate of progression between fibrosis stages. The chapter concludes with a discussion of the estimates produced by the application of these methods.

8.2 Approaches to Model the Natural History of Chronic Hepatitis C

The structure of a model should reflect the natural history of CHC. As a result, important disease stages and transitions should be included. The models that have been used to represent the natural history of CHC usually include as stages the onset of infection and serious sequelae such as cirrhosis, HCC, and death. Depending on the research question, they might include other stages too (e.g., liver transplantation) or expand stages to sub-stages (e.g., cirrhosis to compensated and decompensated cirrhosis). What differentiates the various models that have been used in CHC is the representation of the long and usually asymptomatic period during which a patient is chronically infected but has not yet developed cirrhosis. Based on this, models can be divided in two broad categories (Fig. 8.2):

1. **CHC-based models**: Models that either describe this period as a single stage ("chronic hepatitis") or divide it in sub-stages based on liver histology (e.g., "mild hepatitis" and "moderate hepatitis") (Fig. 8.2a, b)
2. **Fibrosis-based models**: Models that divide the period until the development of cirrhosis in sub-stages based on METAVIR fibrosis stages: F0-no fibrosis;

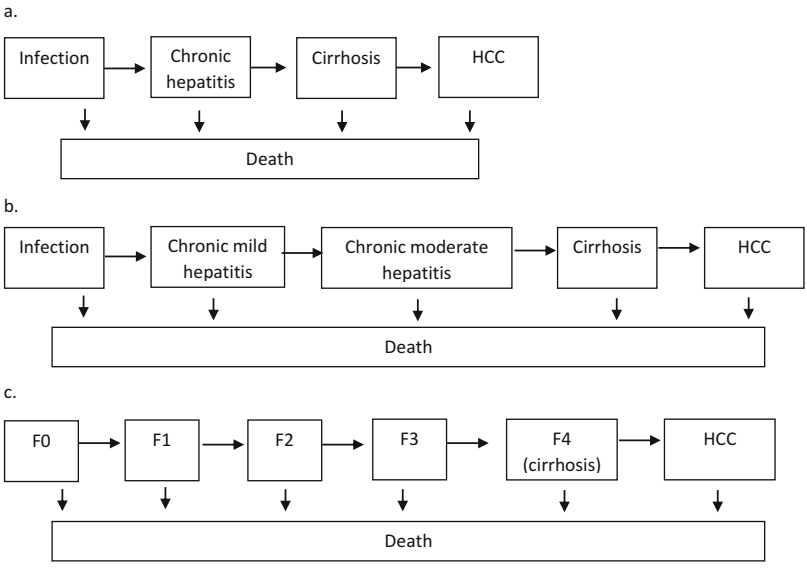


Fig. 8.2 Possible models to represent the natural history of chronic hepatitis C according to the choice of disease stages: (**a**) CHC model where the period before the development of cirrhosis is represented as a single stage, (**b**) CHC model where the period before the development of cirrhosis is divided in sub-stages based on liver histology, (**c**) fibrosis stage model where progression to cirrhosis is modeled using fibrosis stages. Additional stages can be incorporated in these models (e.g., decompensated cirrhosis, transplantation, non-liver and liver-related death, etc.)

F1-fibrous expansion of portal tracts with or without septa; F2-occasional portal to portal bridging; F3-marked bridging fibrosis; and F4-cirrhosis (Fig. 8.2c)

Once a choice about disease stages is made, it is easy to determine which transitions are allowed between these stages. The more stages and transitions incorporated to a model, the more assumptions on transition probabilities should be made. Choosing the appropriate probabilities is crucial as this choice will affect model estimates, such as the time to develop cirrhosis, and model projections of the disease burden. In models of liver disease progression, authors usually perform literature review to obtain estimates for the progression rates between serious sequelae (e.g., from cirrhosis to HCC or from HCC to death). This has been covered in previous chapters and will not be discussed further. Here, we will focus on methods that have been used to obtain appropriate transition probabilities during the asymptomatic period of chronic hepatitis C until the occurrence of cirrhosis.

8.3 CHC-Based Models

CHC-based models were the models of choice in the early publications. One of the first models that have been used to represent the natural history of HCV infection and to project the future disease burden at a population level was proposed by Deuffic et al. [1]. These authors used a model similar to that of Fig. 8.2a where patients who become infected transition to chronic hepatitis and then to serious sequelae (cirrhosis, HCC, death). To calculate the number of patients at the initial stage of their model ("infection"), they first reconstructed the incidence of HCV infection in France up to 1990 and assumed a lower and stable incidence for the subsequent years, as a result of screening of blood donations. Transition probabilities between disease stages were obtained through literature research with the exception of the transition from chronic hepatitis to cirrhosis. A novelty of the model at that time was that the authors recognized the uncertainty concerning the annual probability of progression from chronic hepatitis C to cirrhosis as well as its dependence on patients' age at infection and gender. Thus, they estimated age- and gender-specific probabilities by fitting the model to available data on HCV-related HCC deaths in France. Similar models where the period before the occurrence of serious sequelae is depicted using a single stage ("chronic hepatitis") have been used by other authors too [3, 23].

Other CHC-based models divide the period where patients are chronically infected and without cirrhosis to sub-stages, such as mild and moderate hepatitis. For example, Wong et al. [8] and Sweeting et al. [7] used a model similar to that of Fig. 8.2b to estimate the future hepatitis C morbidity and mortality in the USA and the UK, respectively. They performed literature research to set the annual probabilities of progression from mild chronic hepatitis to moderate and from moderate to cirrhosis. Hutchinson et al. [15] also used a similar model to estimate

the current burden as well as to project the future disease burden among people who inject drugs (PWID) in Scotland.

8.4 Fibrosis-Based Models

In fibrosis-based models, progression to cirrhosis is modeled though METAVIR fibrosis stages (Fig. 8.2c). They soon replaced the initial CHC models and are now extensively used. However, they require estimates on the probability of progression from one fibrosis stage to another. Poynard et al. [24] were the first who provided estimates on liver fibrosis progression and opened the way to model the natural history of CHC through fibrosis stages. Subsequent publications used this approach to project the disease burden, often taking into account the effect of treatment [2, 4–6, 11, 19].

The probability of progression between successive fibrosis stages can be obtained from the estimated annual rate of fibrosis progression, i.e., the number of fibrosis units that a patient progresses per year. As soon as estimates on the annual fibrosis progression rate (**FPR**) are available, the probability of transition can be calculated as follows: $P = 1 - \exp^{-\text{Fibrosis progression rate}}$. There are two approaches to model fibrosis progression (Fig. 8.3):

1. **Constant FPR**: this approach is based on the assumption that fibrosis progresses linearly. Thus, the same rate between consecutive fibrosis stages applies, and a single estimate of FPR is required.
2. **Stage-specific FPR:** under this approach, the rate of fibrosis progression may be nonlinear, and stage-specific transition rates are required, one for each of the transitions F0 → F1, F1 → F2, F2 → F3, and F3 → F4.

Depending on the available data, there are alternative methods to obtain estimates of progression rates. Obviously, the task is much easier under the assumption of linearity as, in this case, a single estimate is desired. Ideally, to estimate the number of fibrosis units that a patient progresses per year, data on a number of patients with

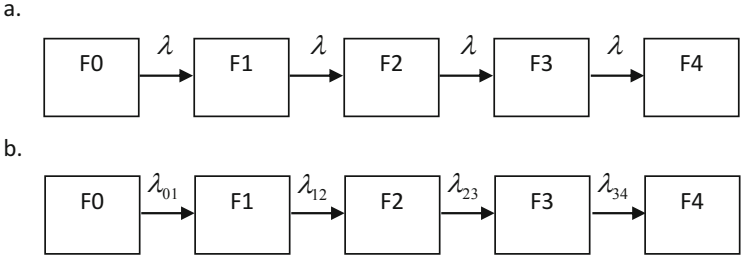
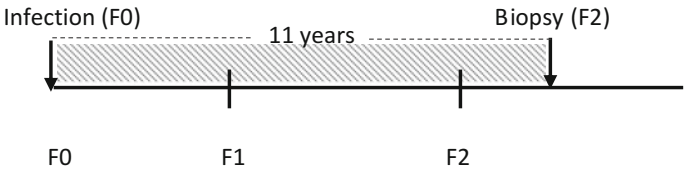


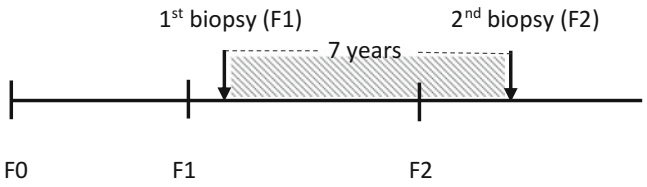
Fig. 8.3 Modeling fibrosis progression assuming: (**a**) linear fibrosis progression (constant progression rate between fibrosis sates), (**b**) nonlinear fibrosis progression (stage-specific progression rates)

a. Indirect method (single biopsy and known duration of infection).



FPR = Stage at biopsy/Duration of infection
= 2/11
= 0.18 unit/year

b. Direct method (multiple biopsies at known times)



FPR = (Stage at 2nd biopsy-Stage at 1st biopsy)/Years between biopsies
= 1/7
= 0.14 units/year

Fig. 8.4 Approaches to estimate the fibrosis progression rate (FPR) depending on the available data. (**a**) Indirect method (single biopsy and known duration of infection). (**b**) Direct method (multiple biopsies at known times)

serial biopsies would be necessary. As this is often not possible, alternative methods based on data with a single biopsy have been used. There are three methods to estimate FPR:

(a) **Indirect method:** It employs patient data with a single biopsy and known duration of infection. It is used when linear fibrosis progression is assumed and results in a single estimate of a constant FPR (Fig. 8.4a).
(b) **Direct method**: It makes use of serial liver biopsies and the interval between two adjacent biopsies. It can be used to estimate constant as well as stage-specific transition rates (Fig. 8.4b).
(c) **Markov maximum likelihood method (MML):** It is an indirect method that allows to obtain stage-specific transition rates when only a single biopsy is available and known duration of infection.

Table 8.1 Alternative methods and data requirements for the estimation of the progression rate between fibrosis stages in models of the natural history of chronic hepatitis C[a]

Assumption on progression rate	Available data	
	Single biopsy and known duration of infection	Serial biopsies
Constant FPR	Indirect method	Direct method
Stage-specific FPR	MML method	Direct method

[a]*FPR* fibrosis progression rate, *MML* Markov maximum likelihood

In Table 8.1, the various approaches used to estimate fibrosis progression are summarized:

Under all these methods, at least one biopsy is required. Although biopsy is the gold standard to assess the level of fibrosis, in recent years, noninvasive methods have been increasingly used to assess hepatic fibrosis. In a meta-analysis, liver stiffness progression rates were estimated based on available data from transient elastography [25]. The authors employed both the direct and the indirect method to estimate constant progression rates.

8.4.1 Estimating Constant FPR (Indirect Method)

Early studies assessing fibrosis progression assumed constant progression rates. The indirect method has been employed extensively to estimate fibrosis progression as it is simple and can be applied even if a single biopsy is available per patient [19, 24, 26–32]. Most studies use the METAVIR fibrosis scoring system. The main idea behind this method is that if there are patient data on METAVIR stage at this single biopsy and on the duration of infection at the time of biopsy, then the number of METAVIR units this patient has progressed over the duration of infection can be inferred. As it can be assumed that patients are at stage F0 at infection, the annual FPR can be defined as the ratio between the fibrosis stage in METAVIR units and the estimated duration of infection at biopsy (Fig. 8.4a), i.e.:

$$FPR = \frac{\text{METAVIR Stage at biopsy} - \text{METAVIR Stage at infection}}{\text{Year of biopsy} - \text{Year of infection}}$$

$$= \frac{\text{METAVIR Stage at biopsy}}{\text{Duration of infection at biopsy}}$$

For example, in the case of a patient with fibrosis stage F2 and an 8-year duration of infection, it can be assumed that the patient progressed 2 METAVIR units (from F0 to F2) in a period of 8 years, i.e., the patient progressed $(2/8) = 0.25$ fibrosis units/year. It is obvious that a constant estimate of fibrosis progression is obtained through this method, i.e., a single estimate for the probability of transitioning between stages F0→F1, F1→F2, F2→F3, and F3→F4. A challenge in the indirect method is that

the duration of infection for each patient is necessary. A common practice is to use the year the patient received transfusion or initiated injecting drug use as the year of infection. In the case of infection through injecting drug use, other authors propose to estimate duration as 2 years after the date of first reported injecting drug use [33].

A constant FPR may be obtained with the direct method, i.e., if there are at least two biopsies per patient (Fig. 8.4b) [32, 34]. In that case, the rate is defined as the ratio between the difference in fibrosis stages between two biopsies and the interval between the two biopsies in years. For example, if a patient progresses from F1 to F3 over a period of 10 years, the rate will be (3–1)/10 = 0.2 units/year. In a meta-analysis of studies on disease progression among PWID, the direct method has been used on data with serial biopsies to estimate a constant fibrosis rate [35].

In both methods, direct or indirect, the mean or the median of the individual rates obtained from patients with available biopsies can be used as the constant FPR to model transition between fibrosis stages. Often, age or age- and sex-specific estimates are obtained to capture the heterogeneity in the progression rates of patients.

8.4.2 Estimating Stage-Specific FPR

Using a constant rate of fibrosis progression in modeling the natural history of CHC implies that fibrosis progression is linear with time and, as a result, the period of time spent in each stage (sojourn time) is the same. Early data questioned this assumption [36], and subsequent analyses supported the view of nonlinearity, i.e., that fibrosis progression advances unevenly over time [30, 37]. The following methods have been used to estimate stage-specific FPR:

8.4.2.1 Estimating Stage-Specific FPR from Serial Biopsy Data

Ideally, the estimation of FPR should be based on data from patients with multiple biopsies as, in this way, it is possible to obtain stage-specific progression rates. However, this method may not be practical as paired biopsies are available in few studies and usually in a small number of patients. Furthermore, it may not be possible to observe the transition of patients between successive fibrosis stages; it might be that patients progress more than one stage in the successive biopsies and, as a result, the question of estimating stage-specific progression rates remains [38].

Deuffic et al. [39] used an alternative approach to overcome the difficulty of applying the direct method on repeated biopsies. They used a Markov model on patients with two biopsies and estimated stage-specific transition rates accounting also for heterogeneity between patients through the introduction of covariates into the model.

8.4.2.2 Estimating Stage-Specific FPR from Single Biopsy Data (MML Method)

As there are few studies available with multiple biopsies per patient, Yi et al. [38] employed the indirect method using Markov maximum likelihood estimation (MML) to estimate stage-specific transition probabilities. Under this approach, a Markov model with fibrosis health states (F0–F4) is created, and the maximum likelihood method is used to estimate stage-specific progression rates. Yi et al. assessed their method on real data as well as through simulations. According to their findings, the MML method predicted fibrosis stage more accurately. The constant method underestimated 30-year cirrhosis rates by up to 40%. Their findings supported the hypothesis that the rates of fibrosis progression vary between stages and suggested that the assumption of constant FPR may lead to substantial inaccuracy in very long-term projections of HCV prognosis. MML has become the method of choice in recent publications estimating fibrosis progression in chronic hepatitis C [27, 35, 40–43].

Apart from the above approaches to estimate stage-specific FPR, Razavi et al. [4] used data on new annual liver cancers and cancer deaths to backcalculate progression rates according to patients' age and gender.

8.5 Using Models of Natural History of Chronic Hepatitis C to Estimate Progression to Cirrhosis and to Project the Burden of Disease

Once the model representing the natural history of chronic hepatitis C is established and appropriate progression rates have been estimated, it is possible to obtain parameters of interest such as estimates concerning time to cirrhosis or projections on HCV-related morbidity and mortality in the following years.

In Table 8.2, estimates of FPR under various estimation methods are presented along with the corresponding estimated time to cirrhosis. When a constant FPR λ is assumed, then the time from infection to cirrhosis T—i.e., from F0 to F4—is estimated as 4 METAVIR units divided by the progression rate, i.e.,

$$T = \frac{4}{\lambda}$$

For example, Poynard et al. applied the indirect method on data from a large cohort of treatment-naïve patients in France with a single biopsy sample and known duration of infection and estimated a median FPR of 0.133 units/year [24]. This estimate corresponds to a median duration of 30 years from infection to cirrhosis (4 units/0.133 units/year). In the case of stage-specific FPR λ_{ij} (i.e., from stage i to the successive stage j), then time to cirrhosis can be estimated using the formula:

Table 8.2 Estimates of FPR and corresponding average time to cirrhosis from meta-analyses

	Estimated FPR (95% CI) in METAVIR units/year	Average time to cirrhosis (years)
Constant FPR (indirect method)		
CHC patients [41]	0.103 (0.098–0.108)	39
HIV/HCV coinfection [42]	0.115 (0.101–0.129)	35
PWID with CHC [35]	0.117 (0.099–0.135)	34
Stage-specific FPR		
CHC patients [41]		
F0 → F1 F1 → F2 F2 → F3 F3 → F4	0.117 (0.104–0.130) 0.085 (0.075–0.096) 0.120 (0.109–0.133) 0.116 (0.104–0.129)	37
HIV/HCV coinfection [42]		
F0 → F1 F1 → F2 F2 → F3 F3 → F4	0.122 (0.098–0.153) 0.115 (0.095–0.140) 0.124 (0.097–0.159) 0.115 (0.098–0.135)	33
PWID with CHC [35]		
F0 → F1 F1 → F2 F2 → F3 F3 → F4	0.128 (0.080–0.176) 0.059 (0.035–0.082) 0.078 (0.056–0.100) 0.116 (0.070–0.161)	46

$$T = \frac{1}{\lambda_{01}} + \frac{1}{\lambda_{12}} + \frac{1}{\lambda_{23}} + \frac{1}{\lambda_{34}}$$

A meta-analysis published in 2008 estimated stage-constant and stage-specific FPR and the corresponding time to cirrhosis [41]. In addition, the available data from different types of epidemiological studies and settings (cross-sectional/retrospective- vs. retrospective-prospective studies, clinical vs. non-clinical settings) were used to estimate stage-specific FPR and explain the heterogeneity in published estimates. The authors applied the MML method, and their results supported nonlinear disease progression, in particular in cross-sectional/retrospective studies and in clinical settings. According to their findings, the rate of progression was generally higher in the initial stages (F0→F1) than the following stages (F1→F2). The highest rate was found in the progression F2→F3. The estimated annual mean stage-specific transition rates were F0→F1 0.117, F1→F2 0.085, F2→F3 0.120, and F3→F4 0.116 units/year (Table 8.2). There was considerable heterogeneity in the obtained estimates depending on the type of study design and setting, as it was also observed by other authors [44, 45]. Overall, time to cirrhosis was 39 years, under the stage-constant FPR, and 37 years under the stage-specific FPR [41].

Smith et al. performed a meta-analysis on hepatitis C progression among PWID and estimated pooled stage-constant and stage-specific FPR [35]. The pooled stage-constant FPR was 0.177 units/year, and the stage-specific rates were F0→F1, 0.128; F1→F2, 0.059; F2→F3, 0.078; and F3→F4, 0.116 units/year (Table 8.2). The

Table 8.3 Estimates of liver stiffness progression rates and corresponding time to cirrhosis based on transient elastography data [25]

	Estimated progression rate (95% CI) in kPa/year	Average time to cirrhosis[a] (years)
Constant liver stiffness progression rate		
All patients	0.181 (0.155–0.206)	40
HIV/HCV-coinfected	0.207 (0.170–0.244)	35

[a]Assuming baseline liver stiffness measurement of 5.33 kPa

average time to cirrhosis was 34 years, under the stage-constant method, and 46 years under the stage-specific method.

Instead of applying these simple formulas to estimate time to cirrhosis, FPR can be used in a Markov model to obtain more refined estimates of the cumulative probability of cirrhosis over time. Markov models simulate disease progression in cohorts of patients and can accommodate the aging of patients and the corresponding mortality in each disease stage [11]. In the case of models where progression rates are dependent on patient' characteristics, simulations of multiple subcohorts of patients—e.g., cohorts classified by gender and age groups—can be performed. For example, Davis et al. estimated the prevalence of cirrhosis at 20 years after the infection to be 2.4% in women and 27.8% in men (assuming age at infection between 31 and 50 years) [11].

It is also possible to estimate time to cirrhosis from liver stiffness progression rates (LSPR) based on liver stiffness assessed by transient elastography [25]. Assuming a cut-off for cirrhosis of 12.5 kPa and a baseline liver stiffness measurement corresponding to healthy individuals ($LS_{Healthy}$) of 5.33 kPa or 4.6 kPa, the time to cirrhosis is estimated as:

$$T = \frac{12.5 - LS_{Healthy}}{LSPR}$$

In Table 8.3, estimates obtained from a meta-analysis on liver stiffness progression rates and the corresponding time to cirrhosis are presented [25]. These authors compared liver stiffness progression rates, based on transient elastography, to FPR, based on biopsy data, and the resulting predicted time to cirrhosis was similar under both approaches. However, there was less consistency for early-stage progression (time to F2). This discrepancy may be attributed to diagnostic performance of transient elastography and possible confounding [25].

Markov models that describe the natural history of chronic hepatitis C can be also use to project the future disease burden. A cohort of patients is assumed, e.g., all newly infected patients in a particular year, and is followed over time as they transition from disease stage F0 to subsequent stages from one year to another with the appropriate progression rates. The model keeps track of what proportion of the cohort is in each stage over time. In this way, it is possible to calculate both the number of patients who would enter into the specific disease stages in a year (annual

incident cases) and the number of patients who would be at the specific disease stages during this year (annual prevalent cases) for a specific time period, e.g., for the next 15 years [1, 3, 7, 8, 11, 19, 23, 43].

The extensive work on the estimation of fibrosis progression and on models describing the natural history of hepatitis C has been leveraged in more recent years to identify treatment strategies that will allow reaching the goal of eliminating viral hepatitis as a public health problem by 2030. Once a model and appropriate transition probabilities are selected, it is easy to incorporate the impact of treatment by, e.g., assuming that disease progression is halted in pre-cirrhotic patients and is slower in those with cirrhosis. In particular, a model with stage-specific FPR has been used to explore the impact of alternative treatment strategies on the future disease burden in 64 countries [21, 46]. Lim et al. proposed an approach to estimate the disease burden and assess the impact of scaling up treatment and prevention in Pakistan which, in addition, takes into account HCV dynamic transmission in the disease progression model [47]. As this chapter focusses on natural history, i.e., disease progression in the absence of treatment, these applications of the models will not be discussed further.

References

1. Deuffic S, Buffat L, Poynard T, Valleron AJ. Modeling the hepatitis C virus epidemic in France. Hepatology. 1999;29:1596–601. https://doi.org/10.1002/hep.510290528.
2. Hatzakis A, et al. The present and future disease burden of hepatitis C virus (HCV) infections with today's treatment paradigm—volume 2. J Viral Hepat. 2015;22(Suppl 1):26–45. https://doi.org/10.1111/jvh.12351.
3. Lehman EM, Wilson ML. Epidemic hepatitis C virus infection in Egypt: estimates of past incidence and future morbidity and mortality. J Viral Hepat. 2009;16:650–8. https://doi.org/10.1111/j.1365-2893.2009.01115.x.
4. Razavi H, et al. The present and future disease burden of hepatitis C virus (HCV) infection with today's treatment paradigm. J Viral Hepat. 2014;21(Suppl 1):34–59. https://doi.org/10.1111/jvh.12248.
5. Salomon JA, Weinstein MC, Hammitt JK, Goldie SJ. Empirically calibrated model of hepatitis C virus infection in the United States. Am J Epidemiol. 2002;156:761–73.
6. Sibley A, et al. The present and future disease burden of hepatitis C virus infections with today's treatment paradigm—volume 3. J Viral Hepat. 2015;22(Suppl 4):21–41. https://doi.org/10.1111/jvh.12476.
7. Sweeting MJ, De Angelis D, Brant LJ, Harris HE, Mann AG, Ramsay ME. The burden of hepatitis C in England. J Viral Hepat. 2007;14:570–6. https://doi.org/10.1111/j.1365-2893.2007.00851.x.
8. Wong JB, McQuillan GM, McHutchison JG, Poynard T. Estimating future hepatitis C morbidity, mortality, and costs in the United States. Am J Public Health. 2000;90:1562–9.
9. Alfaleh FZ, et al. Strategies to manage hepatitis C virus infection disease burden—volume 3. J Viral Hepat. 2015;22(Suppl 4):42–65. https://doi.org/10.1111/jvh.12474.
10. Bennett WG, Inoue Y, Beck JR, Wong JB, Pauker SG, Davis GL. Estimates of the cost-effectiveness of a single course of interferon-alpha 2b in patients with histologically mild chronic hepatitis C. Ann Intern Med. 1997;127:855–65.
11. Davis GL, Alter MJ, El-Serag H, Poynard T, Jennings LW. Aging of hepatitis C virus (HCV)-infected persons in the United States: a multiple cohort model of HCV prevalence and disease

progression. Gastroenterology. 2010;138:513–21e511–516. https://doi.org/10.1053/j.gastro.2009.09.067.
12. El Saadany S, Coyle D, Giulivi A, Afzal M. Economic burden of hepatitis C in Canada and the potential impact of prevention. Results from a disease model. Eur J Health Econ. 2005;6:159–65. https://doi.org/10.1007/s10198-004-0273-y.
13. Gane E, et al. Strategies to manage hepatitis C virus (HCV) infection disease burden—volume 2. J Viral Hepat. 2015;22(Suppl 1):46–73. https://doi.org/10.1111/jvh.12352.
14. Gountas I, Sypsa V, Papatheodoridis G, Souliotis G, Razavi H, Hatzakis A. Is elimination of HCV possible in a country with low diagnostic rate and moderate HCV prevalence?: The case of Greece. J Gastroenterol Hepatol. 2017;32:466–72. https://doi.org/10.1111/jgh.13485.
15. Hutchinson SJ, Bird SM, Goldberg DJ. Modeling the current and future disease burden of hepatitis C among injection drug users in Scotland. Hepatology. 2005;42:711–23. https://doi.org/10.1002/hep.20836.
16. Kabiri M, Jazwinski AB, Roberts MS, Schaefer AJ, Chhatwal J. The changing burden of hepatitis C virus infection in the United States: model-based predictions. Ann Intern Med. 2014;161:170–80. https://doi.org/10.7326/M14-0095.
17. Meijerink H, et al. Modelling the burden of hepatitis C infection among people who inject drugs in Norway, 1973–2030. BMC Infect Dis. 2017;17:541. https://doi.org/10.1186/s12879-017-2631-2.
18. Sypsa V, et al. Future trends of HCV-related cirrhosis and hepatocellular carcinoma under the currently available treatments. J Viral Hepat. 2005;12:543–50. https://doi.org/10.1111/j.1365-2893.2005.00588.x.
19. Sypsa V, et al. Reconstructing and predicting the hepatitis C virus epidemic in Greece: increasing trends of cirrhosis and hepatocellular carcinoma despite the decline in incidence of HCV infection. J Viral Hepat. 2004;11:366–74. https://doi.org/10.1111/j.1365-2893.2004.00517.x.
20. Ward T, et al. Tackling the burden of the hepatitis C virus in the UK: characterizing and assessing the clinical and economic consequences. Public Health. 2016;141:42–51. https://doi.org/10.1016/j.puhe.2016.08.002.
21. Wedemeyer H, et al. Strategies to manage hepatitis C virus (HCV) disease burden. J Viral Hepat. 2014;21(Suppl 1):60–89. https://doi.org/10.1111/jvh.12249.
22. Dore GJ, Freeman AJ, Law M, Kaldor JM. Natural history models for hepatitis C-related liver disease: different disease progression parameters for different settings. Antivir Ther. 2003;8:365–72.
23. Griffiths J, Nix B. Modeling the hepatitis C virus epidemic in France using the temporal pattern of hepatocellular carcinoma deaths. Hepatology. 2002;35:709–15. https://doi.org/10.1053/jhep.2002.31258.
24. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. Lancet. 1997;349:825–32.
25. Erman A, et al. Estimating chronic hepatitis C prognosis using transient elastography-based liver stiffness: a systematic review and meta-analysis. J Viral Hepat. 2017;25:502–13. https://doi.org/10.1111/jvh.12846.
26. Benhamou Y, et al. Liver fibrosis progression in human immunodeficiency virus and hepatitis C virus coinfected patients. Multivirc Group Hepatol. 1999;30:1054–8. https://doi.org/10.1002/hep.510300409.
27. Bochud PY, et al. Genotype 3 is associated with accelerated fibrosis progression in chronic hepatitis C. J Hepatol. 2009;51:655–66. https://doi.org/10.1016/j.jhep.2009.05.016.
28. Hezode C, et al. Daily cannabis smoking as a risk factor for progression of fibrosis in chronic hepatitis C. Hepatology. 2005;42:63–71. https://doi.org/10.1002/hep.20733.
29. Hissar SS, et al. Natural history of hepatic fibrosis progression in chronic hepatitis C virus infection in India. J Gastroenterol Hepatol. 2009;24:581–7. https://doi.org/10.1111/j.1440-1746.2008.05649.x.

30. Matsumura H, Moriyama M, Goto I, Tanaka N, Okubo H, Arakawa Y. Natural course of progression of liver fibrosis in Japanese patients with chronic liver disease type C--a study of 527 patients at one establishment. J Viral Hepat. 2000;7:268–75.
31. Reiberger T, et al. HIV-HCV co-infected patients with low CD4+ cell nadirs are at risk for faster fibrosis progression and portal hypertension. J Viral Hepat. 2010;17:400–9. https://doi.org/10.1111/j.1365-2893.2009.01197.x.
32. Sobesky R, et al. Modeling the impact of interferon alfa treatment on liver fibrosis progression in chronic hepatitis C: a dynamic view. Multivirc Group Gastroenterol. 1999;116:378–86.
33. Combellick J, Smith DJ, Jordan AE, Hagan H. Hepatitis C virus disease progression in people who inject drugs: protocol for a systematic review and meta-analysis. JMIR Res Protoc. 2015;4:e68. https://doi.org/10.2196/resprot.4518.
34. Bonnard P, Lescure FX, Amiel C, Guiard-Schmid JB, Callard P, Gharakhanian S, Pialoux G. Documented rapid course of hepatic fibrosis between two biopsies in patients coinfected by HIV and HCV despite high CD4 cell count. J Viral Hepat. 2007;14:806–11. https://doi.org/10.1111/j.1365-2893.2007.00874.x.
35. Smith DJ, Combellick J, Jordan AE, Hagan H. Hepatitis C virus (HCV) disease progression in people who inject drugs (PWID): a systematic review and meta-analysis. Int J Drug Policy. 2015;26:911–21. https://doi.org/10.1016/j.drugpo.2015.07.004.
36. Datz C, et al. The natural course of hepatitis C virus infection 18 years after an epidemic outbreak of non-A, non-B hepatitis in a plasmapheresis centre. Gut. 1999;44:563–7.
37. Poynard T, Ratziu V, Charlotte F, Goodman Z, McHutchison J, Albrecht J. Rates and risk factors of liver fibrosis progression in patients with chronic hepatitis C. J Hepatol. 2001;34:730–9.
38. Yi Q, Wang PP, Krahn M. Improving the accuracy of long-term prognostic estimates in hepatitis C virus infection. J Viral Hepat. 2004;11:166–74.
39. Deuffic-Burban S, Poynard T, Valleron AJ. Quantification of fibrosis progression in patients with chronic hepatitis C using a Markov model. J Viral Hepat. 2002;9:114–22.
40. Eslam M, et al. Interferon-lambda rs12979860 genotype and liver fibrosis in viral and non-viral chronic liver disease. Nat Commun. 2015;6:6422. https://doi.org/10.1038/ncomms7422.
41. Thein HH, Yi Q, Dore GJ, Krahn MD. Estimation of stage-specific fibrosis progression rates in chronic hepatitis C virus infection: a meta-analysis and meta-regression. Hepatology. 2008;48:418–31. https://doi.org/10.1002/hep.22375.
42. Thein HH, Yi Q, Dore GJ, Krahn MD. Natural history of hepatitis C virus infection in HIV-infected individuals and the impact of HIV in the era of highly active antiretroviral therapy: a meta-analysis. AIDS. 2008;22:1979–91. https://doi.org/10.1097/QAD.0b013e32830e6d51.
43. Thein HH, Yi Q, Heathcote EJ, Krahn MD. Prognosis of hepatitis C virus-infected Canadian post-transfusion compensation claimant cohort. J Viral Hepat. 2009;16:802–13. https://doi.org/10.1111/j.1365-2893.2009.01136.x.
44. Freeman AJ, et al. Estimating progression to cirrhosis in chronic hepatitis C virus infection. Hepatology. 2001;34:809–16. https://doi.org/10.1053/jhep.2001.27831.
45. Sweeting MJ, et al. Estimated progression rates in three United Kingdom hepatitis C cohorts differed according to method of recruitment. J Clin Epidemiol. 2006;59:144–52. https://doi.org/10.1016/j.jclinepi.2005.06.008.
46. Chen DS, et al. Strategies to manage hepatitis C virus infection disease burden-Volume 4. J Viral Hepat. 2017;24(Suppl 2):44–63. https://doi.org/10.1111/jvh.12759.
47. Lim AG, et al. Curbing the hepatitis C virus epidemic in Pakistan: the impact of scaling up treatment and prevention for achieving elimination International. J Epidemiol. 2018;47:550–60. https://doi.org/10.1093/ije/dyx270.

Prevention: Secondary Prevention and Screening

9

Vana Sypsa

9.1 Introduction

The asymptomatic nature and long latent phase of HCV infection results in considerable under-diagnosis. Globally, only 20% of HCV-infected persons have been diagnosed (2015 estimate) [1]. One of the targets set in WHO Global Health Sector Strategy on Viral Hepatitis 2016–2021 is to increase the proportion of diagnosed patients from 20% in 2015 to 30% in 2020 and 90% in 2030 [2]. This will improve access to treatment and will allow to reach the target of reducing HCV-related mortality by 2030.

Increasing diagnosis means that a growing number of asymptomatic chronically infected patients will have to be identified in the following years. This can be achieved through screening. The term "screening" refers to "The presumptive identification of unrecognized disease or defect by the application of tests, examinations, or other procedures which can be applied rapidly" [3]. Screening is a secondary prevention measure; it does not prevent disease, but it identifies asymptomatic individuals at an earlier stage than if they waited for the occurrence of symptoms. Thus, it aims at the window between the onset of the disease and the occurrence of clinical symptoms. A basic assumption of screening is that earlier diagnosis benefits the patient; treatment may be more effective when provided at an earlier stage of the disease, and, as a result, disease progression may slow or halt. In the case of infectious diseases, earlier diagnosis results to benefit not only at the individual level but also at the population level as awareness and treatment may prevent further transmission.

Screening as a secondary prevention measure should not be confused with unlinked anonymous testing; the latter is a surveillance method used to monitor

V. Sypsa (✉)
Department of Hygiene, Epidemiology and Medical Statistics, Medical School, National and Kapodistrian University of Athens, Athens, Greece
e-mail: vsipsa@med.uoa.gr

A. Hatzakis (ed.), *Hepatitis C: Epidemiology, Prevention and Elimination*,
https://doi.org/10.1007/978-3-030-64649-3_9

the epidemiology of a disease and cannot be used to increase diagnosis and treatment as test results are irreversibly unlinked from information identifying the individual.

Screening for hepatitis C can help to reach both impact targets set by the WHO concerning the reduction in incidence and mortality. Diagnosed asymptomatic individuals may get cured, and, as a result, they have lower risk of HCV-related death; in addition, they cannot transmit HCV to other people, and incidence is anticipated to decline. However, not all diseases are amenable to screening; certain characteristics have to be fulfilled. In this chapter, we will discuss why HCV is an appropriate disease for screening programs. In addition, there are important questions concerning who should be tested and how. Various screening approaches are proposed, and the selection of the appropriate strategy depends on the desired objective, the epidemiology of the disease in the population, cost and available resources. An overview of screening strategies will be presented in this chapter. In addition, there will be a discussion of ethical issues and of challenges in implementing an efficient screening program, in particular those related to the availability of resources for testing and the ability to improve all steps of the cascade of care once a patient has been identified.

9.2 Rationale for Performing Screening for Hepatitis C

Wilson and Jungner proposed ten criteria for the evaluation of screening programs [4] (Table 9.1). Chronic hepatitis C has the epidemiological and clinical characteristics that make it an appropriate disease for screening. First of all, it is a serious disease. Viral hepatitis is considered as one of the leading causes of death and disability worldwide [5], and 48% of hepatitis-related deaths are attributed to HCV [2]. Its natural history has been extensively studied in various settings with several study designs: cross-sectional, retrospective and prospective studies have been performed in clinical and non-clinical settings [6, 7]. It has a detectable long preclinical phase with an estimated average time to cirrhosis of 39 years [6].

Apart from the characteristics related to the disease itself, there are additional arguments for screening for HCV. Treatment with interferon-free direct-acting antivirals (DAAs) is acceptable to patients due to its high efficacy, short duration and favourable safety profile. Sustained virological response is associated with reduced risk of fibrosis progression, liver-related and all-cause mortality [8]. In addition, although DAAs have high efficacy in all disease stages, they are more effective in patients without cirrhosis than in patients with cirrhosis [9–11]. There are suitable tests to assess whether people have been exposed to the virus (enzyme immunoassays and rapid diagnostic tests) which are acceptable to the population and have high sensitivity and specificity.

Table 9.1 Rationale for performing screening for chronic hepatitis C

Wilson and Jungner criteria for screening [4]	Chronic hepatitis C
1. The condition should be an important health problem	It leads to serious sequelae (cirrhosis, hepatocellular carcinoma, death) and is considered as one of the leading causes of death and disability worldwide [5]
2. There should be a recognisable latent or early symptomatic stage	Its natural history is characterised by a long latent phase (39 years on average from infection to cirrhosis) [6]
3. The natural history of the condition, including development from latent to declared disease, should be adequately understood	The natural history of chronic hepatitis C has been extensively studied in various settings with several study designs [6, 7]
4. There should be an accepted treatment for patients with recognised disease	Treatment with DAAs is acceptable to patients due to its high efficacy, short duration and favourable safety profile
5. There should be a suitable test or examination that has a high level of accuracy	Enzyme immunoassays (EIA) and rapid diagnostic tests (RDT) are available with high sensitivity and specificity. Blood samples (collected either by capillary fingerstick or by venipuncture) and oral fluid can be used
6. The test should be acceptable to the population	
7. There should be an agreed policy on whom to treat as patients	It depends on the setting where screening is implemented
8. Facilities for diagnosis and treatment should be available	
9. The cost of screening (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole	
10. Screening should be a continuing process and not a "once and for all" project	

9.3 Key Drivers of Cost-Effectiveness of HCV Screening

The formulation of a screening strategy is contingent on its cost-effectiveness, i.e. on the balance of cost and benefits. Benefits are measured in life years gained or quality-adjusted life years gained through early diagnosis and treatment. The following key drivers of cost-effectiveness have been identified for HCV screening [12, 13]:

1. **Prevalence of HCV and degree of concentration of epidemic:** The higher the prevalence in the population targeted by the screening program, the higher the number of cases detected. If there are specific risk groups affected by HCV in a population, then targeted screening is more cost-effective.
2. **Efficacy of treatment:** When the efficacy of treatment increases, the benefits measured by life years or quality-adjusted life years gained increase as well.

3. **Availability of treatment and linkage to care:** If there are restrictive policies on access to treatment, then screening becomes less cost-effective. Similarly, failure to link identified cases to care and treatment reduces the value of screening.
4. **Quality of life with early stage HCV infection:** The assumption concerning normal or low quality of life in early stage infection affects the cost-effectiveness of screening. As expected, the lower the quality of life with early stage HCV infection, the higher the value of screening.
5. **Fibrosis stage and fibrosis progression:** The average disease stage of the population that is being screened is important. As HCV-related morbidity and mortality is associated with advanced fibrosis stage, a greater gain from HCV screening and cure is anticipated in populations with advanced average fibrosis stage and/or faster fibrosis progression.
6. **Cost of testing and treatment:** The cost of testing has little impact when the question is about who should be screened. On the other hand, as the cost of treatment decreases, the cost-effectiveness of screening becomes higher.

9.4 Screening Approaches

In general, the performance of screening depends on the accuracy of the screening test and its predictive value. The former is measured through sensitivity and specificity, i.e. the ability of the test to identify correctly those who have the disease and those who do not have the disease. The positive predictive value is the probability that a person who reacts positively to the test actually has the disease. This is influenced not only by the sensitivity and specificity of the test but also by the prevalence of the disease in the population that is being screened; unless the specificity of a test is perfect, the positive predictive value decreases with decreasing prevalence. Thus, a common approach in screening programs is to test people who are at higher risk of the disease as this increases the positive predictive value and the yield, i.e. the number of cases detected.

Screening approaches vary and depend on the type of HCV epidemic in the target population and on the desirable increase in the proportion of diagnosed persons. Globally, HCV epidemics are a mix of the following epidemic components [13]:

1. **Infection associated with high-risk behaviours:** People who inject drugs (PWID) constitute a population group with high HCV prevalence worldwide [14]. Other populations with increased transmission are prisoners, as often incarceration is associated with drug-related offences, sex workers and men who have sex with men.
2. **Birth cohort epidemic:** This type of epidemic results from generalised exposure of the population to a source that has been identified and removed. As a result, higher HCV prevalence is found in specific age groups, for example, among older people who were likely to have been infected before the introduction of screening of blood supply.

3. **Generalised epidemic:** In this type of epidemic, there is widespread exposure of the population to HCV across all age groups. It usually results from iatrogenic exposure and results in high prevalence in the general population.

Knowledge of the type of epidemic can guide the selection of the appropriate screening strategy. The main screening approaches in HCV infection are the following [13]:

1. **Focused testing.**
   (a) **ALT-based screening:** HCV testing for persons with elevated alanine aminotransferase (ALT) levels
   (b) **Risk-based screening:** HCV testing for persons who are part of a population with high HCV seroprevalence or who have a history of exposure and/or high-risk behaviours for HCV infection
2. **Birth cohort screening:** HCV testing of people who are born within a specific range of years (birth cohorts).
3. **General population:** HCV testing in the general population—this is recommended in areas of intermediate to high seroprevalence in the general population ($\geq$2% or $\geq$5%).

Focused screening is implemented to populations where a high HCV prevalence is anticipated as a result of risk exposures, behaviours and conditions (Table 9.2) [15]. It includes HCV testing for persons with elevated ALT levels (ALT-based screening) which is the approach traditionally used by healthcare providers. The prevalence of HCV infection among individuals with elevated ALT levels may be several-fold higher compared with those with normal ALT levels, and, as a result, the yield of this approach is favourable. For example, according to data from 19,000 adults in the USA who completed the National Health and Nutrition Examination Survey, HCV prevalence was 8.4% vs. 1.1% among persons with ALT levels $\geq$40 U/L and <40 U/L, respectively [16]. Similarly, high prevalence is anticipated in specific risk groups such as PWID, people on haemodialysis, etc. (Table 9.2). However, it is uncertain whether these approaches of focused testing in most affected populations are appropriate to achieve a high proportion of diagnosis in HCV. From an assessment of ALT-based screening in the population of the USA, it was estimated that it can lead to the diagnosis of 50% of patients, which is far lower compared to the target of 90% set by the WHO [16]. Wolffram et al. reported that screening based on elevated ALT levels in the primary care setting in Germany would identify 29% of previously undiagnosed anti-HCV positive persons and 55% of chronically infected cases [17]. The combination of at least one of three HCV risk factors (PWID, blood transfusion before 1992 and immigration) or elevated ALT levers would double the number of tested persons and would identify 59% of previously undiagnosed anti-HCV positive person and 83% of chronically infected cases [17].

Birth cohort screening is suggested as a preferable alternative to focused screening as it can identify a much higher number of persons with HCV infection.

Table 9.2 Risk exposures, behaviours and conditions included in focused screening (AASLD recommendations) [15]

Risk exposures
Long-term haemodialysis (ever)
Percutaneous/parenteral exposures in an unregulated setting
Healthcare, emergency medical and public safety workers after needlesticks, sharps or mucosal exposures to HCV-infected blood
Children born to HCV-infected women
Prior recipients of transfusions or organ transplants, including persons who: (1) Were notified that they received blood from a donor who later tested positive for HCV infection (2) Received a transfusion of blood or blood components or underwent an organ transplant before July 1992 (3) Received clotting factor concentrates produced before 1987
Persons who were ever incarcerated
Risk behaviours
Injection drug use (current or ever, including those who inject once)
Intranasal illicit drug use
Other
HIV infection
Sexually active persons about to start pre-exposure prophylaxis for HIV
Unexplained chronic liver disease and chronic hepatitis, including elevated alanine aminotransferase levels
Solid organ donors (deceased and living)

Recommendations for HCV screening in the USA include one-time HCV testing for persons born between 1945 and 1965, regardless of country of birth, without prior ascertainment of risk [15]. Screening of the 1945–1965 birth cohort has the potential to diagnose 77% of HCV infected cases compared to 50% under the ALT-based strategy, i.e. about one million more anti-HCV positive people [16]. In another study in the USA, it was estimated that risk-based screening could identify 21% of previously undiagnosed cases vs. 86% under birth cohort screening [18]. As expected, this approach demands more resources compared to risk-based screening, but it is more cost-effective, even under the lower treatment acceptance and efficacy of interferon-based therapy [19]. From the implementation of birth cohort screening in the USA since 2012, two weaknesses have been identified: the rate of screening of the target population and the rate of linking identified patients to care remain low. From large insurance databases, it was estimated that the proportion of those screened for HCV increased from approximately 1.8% of the target population in 2011 to only 3.3% in 2014 [20]. The proportion of screening in baby boomers ranges from 0.09% in emergency departments to 18% in primary care [21]. For this reason, alternative approaches have been proposed to boost the coverage of birth cohort screening, such as electronic health record alerts in primary care [22]. In addition, it is suggested that birth cohort screening should include effective linkage-to-care models to ensure the continuity of care [23]. New York enacted a law that requires healthcare providers to offer HCV screening to every individual born during

1945–1965 who receives health services as an inpatient in hospital or who receives primary care services in an outpatient department or in a diagnostic and treatment centre. In addition, they should offer follow-up healthcare or refer to a healthcare provider who can provide follow-up healthcare, including a hepatitis C diagnostic test (NY Pub Health L § 2171. Required offering of hepatitis C screening testing [24]).

General population screening is an approach that has received attention in the recent years, in view of the target of 90% diagnosis in HCV infection by 2030. In Egypt, where the prevalence of HCV is high in the general population, this type of screening has been shown to be cost-effective even if pegylated interferon is used [25]. In Switzerland, it is estimated that the number of persons screened to identify 1 new viraemic HCV case was 90 and 159, for birth cohort and general population screening, respectively, with obvious consequences in the cost per case detected [26]. One-time screening of the US general population in the era of effective treatment was found to be cost-saving compared to birth cohort or risk-based screening [27]. Given the high extent of such a screening approach, it is recommended to use existing testing opportunities or programs for its implementation [13].

Various studies have assessed the efficacy of HCV screening in emergency departments (ED) [28–31]. Galbraith et al. [30] reported a high prevalence of unrecognised HCV infection in baby boomers presenting in ED and concluded that this setting is important for high-impact HCV screening. In another study, the authors suggested a practice of universal one-time testing in high-risk urban ED settings as they have found that one quarter of infections in an urban ED in the USA would remain undiagnosed if birth cohort and risk-based screenings were offered [31]. On the other hand, subsequent linkage to HCV care may be low, and additional strategies should be implemented for optimal linkage in emergency departments [29].

9.5 How to Implement Screening: Integrated vs. Non-integrated Programs

Once the appropriate screening strategy is selected, an important question is how it is going to be implemented. One option is to integrate screening programs within already existing healthcare services and facilities (STD clinics, HIV/STD service providers, sexual and reproductive health clinics, emergency departments, community centres, prisons, health clinics, primary care, etc.). Another option is to perform non-integrated screening programs, i.e. programs exclusively set up for screening (community health clinics, private practice offices, outreach community screening, city programs, schools, etc.).

Integrated screening programs present some important advantages; they do not have to attract the target population for screening, they can use a facility that is familiar to the public, and long-term sustainability and continuity is ensured as they facilitate continuous screening at relatively low cost [32]. Integration is anticipated

to make screening programs more cost-effective because fewer additional resources would be required [33]. On the other hand, they might not be successful in reaching populations other than those who have a reason to visit such facilities [32]. Integration of screening in primary care may be more appropriate to reach a wide population and to scale up HCV testing [34].

Non-integrated programs may attract a different risk population that otherwise would not be screened. However, they are highly labour-intensive and they are often performed for a limited period. As a result, they are not sustainable. To increase their cost-effectiveness, it is suggested to screen for other diseases simultaneously, when risk groups overlap [32].

Overall, both approaches are useful and can be used complementary to each other.

9.6 How to Test for HCV in Screening Programs

The ideal screening test should be inexpensive, safe, easy to administrate and sensitive, i.e. capable of detecting all true positives. Screening of HCV infection is usually performed through a single serological assay that detects antibodies to HCV. Usually, laboratory-based enzyme immunoassays (EIA) are used, but due to advances in HCV detection technology, the use of rapid diagnostic tests (RDT) is growing. RDT for HCV infection are suitable for settings with limited access to laboratory services and for hard-to-reach populations, and they have high sensitivity and specificity across populations [35]. However, as there is considerable variability in clinical performances of available tests depending on the manufacturer, it is important to select prequalified RDT that have clinical sensitivity and specificity compared to standard EIA assays [36–38]. Both EIA and RDT assays are simple and low cost [12].

Dried blood spot (DBS) sampling is a method to collect whole blood specimens. The blood can be collected either by capillary fingerstick or by venipuncture, and the sample is transferred onto filter paper. This approach can increase HCV test uptake in specific settings or populations (e.g. persons with poor venous access), and, in addition, the collected specimens can be easily transported [13].

Screening programs cannot be successful if they do not combine linkage to care for identified cases. HCV may completely resolve during the acute phase of infection without treatment in around 20–30% of infected cases. These cases will test positive in the assays detecting antibodies. In addition, a growing number of patients are anticipated to receive treatment in the following years and get cured. In persons who were successfully treated in the past but continue to practice high-risk behaviours, such as PWID, a positive antibody test is not meaningful as it cannot differentiate between past resolved infection and reinfection. For all these reasons, the use of a nucleic acid testing (NAT) is recommended as the preferred strategy to diagnose viraemic HCV infection, following a positive HCV antibody serological test. A core HCV antigen assay has comparable clinical sensitivity and is an alternative to NAT to diagnose viraemic infection [13]. It has been proposed as assay to be used in an

ideal future algorithm for one-step screening as only one test would be required for both screening and diagnosis of HCV [39].

9.7 Ethical Issues in HCV Screening Programs

The WHO has outlined five principles for hepatitis testing in all settings (5 Cs): consent, confidentiality, counselling, correct test results and connection (linkage to prevention, treatment and care services) [13].

Testing for hepatitis C must be voluntary, and informed consent has to be sought. The consent process may be "opt-in" or "opt-out". In "opt-out" screening, HCV testing is performed after notifying the person that the test will be done; consent is inferred unless the person declines. In "opt-in" screening, patients are informed that tests are available but are not tested unless they request to be tested. In vulnerable populations, the "opt-out" approach has received criticism. For example, in prisoner populations, it may be questionable whether consent for HCV testing is truly voluntary and free from coercion; instead, provider-initiated "opt-in" screening is suggested [40]. However, "opt-in" is anticipated to reduce the uptake of HCV testing, as it has been shown in the case of HIV [41].

Testing should be accessible to the populations most affected in an environment that minimises stigma and discrimination. Client confidentiality is important for screening. Furthermore, screening should be considered as part of the continuum of care. People have to be reached, tested and informed of their test result. In addition, they have to be enrolled to care, initiate treatment and remain on treatment. Thus, linkage to treatment, care and support services should be integral part of a screening program in order to maximise both individual and public health benefits. Finally, screening and treatment should be in balance with primary prevention.

References

1. WHO. Global Hepatitis Report 2017. Geneva: World Health Organization; 2017.
2. WHO. Global Health Sector Strategy on viral hepatitis 2016–2021. Towards ending viral hepatitis. 2016. http://apps.who.int/iris/bitstream/10665/246177/1/WHO-HIV-2016.06-eng.pdf?ua=1.
3. Porta M. A dictionary of Epidemiology. 6th ed. Oxford: Oxford University Press; 2014.
4. Wilson J, Jungner G. Principles and practice of screening for disease. 1968.
5. Stanaway JD, et al. The global burden of viral hepatitis from 1990 to 2013: findings from the Global Burden of Disease Study 2013. Lancet. 2016;388:1081–8. https://doi.org/10.1016/S0140-6736(16)30579-7.
6. Thein HH, Yi Q, Dore GJ, Krahn MD. Estimation of stage-specific fibrosis progression rates in chronic hepatitis C virus infection: a meta-analysis and meta-regression. Hepatology. 2008;48:418–31. https://doi.org/10.1002/hep.22375.
7. Freeman AJ, et al. Estimating progression to cirrhosis in chronic hepatitis C virus infection. Hepatology. 2001;34:809–16. https://doi.org/10.1053/jhep.2001.27831.
8. Terrault NA, Hassanein TI. Management of the patient with SVR. J Hepatol. 2016;65:S120–9. https://doi.org/10.1016/j.jhep.2016.08.001.

9. Falade-Nwulia O, Suarez-Cuervo C, Nelson DR, Fried MW, Segal JB, Sulkowski MS. Oral direct-acting agent therapy for hepatitis C virus infection: a systematic review. Ann Intern Med. 2017;166:637–48. https://doi.org/10.7326/M16-2575.
10. Fathi H, Clark A, Hill NR, Dusheiko G. Effectiveness of current and future regimens for treating genotype 3 hepatitis C virus infection: a large-scale systematic review. BMC Infect Dis. 2017;17:722. https://doi.org/10.1186/s12879-017-2820-z.
11. Ji F, et al. Systematic review with meta-analysis: effectiveness and tolerability of interferon-free direct-acting antiviral regimens for chronic hepatitis C genotype 1 in routine clinical practice in Asia. Aliment Pharmacol Therapeut. 2018;47:550–62. https://doi.org/10.1111/apt.14507.
12. Morgan JR, Servidone M, Easterbrook P, Linas BP. Economic evaluation of HCV testing approaches in low and middle income countries. BMC Infect Dis. 2017;17:697. https://doi.org/10.1186/s12879-017-2779-9.
13. WHO. Guidelines on hepatitis B and C testing. Geneva: World Health Organization; 2017.
14. Degenhardt L, et al. Global prevalence of injecting drug use and sociodemographic characteristics and prevalence of HIV, HBV, and HCV in people who inject drugs: a multistage systematic review. Lancet Glob Health. 2017;5:e1192–207. https://doi.org/10.1016/S2214-109X(17)30375-3.
15. AASLD, IDSA. HCV guidance: recommendations for testing, managing, and treating hepatitis C. 2017 (last updated: 21 September 2017).
16. Smith BD, Yartel AK. Comparison of hepatitis C virus testing strategies: birth cohort versus elevated alanine aminotransferase levels. Am J Prev Med. 2014;47:233–41. https://doi.org/10.1016/j.amepre.2014.05.011.
17. Wolffram I, et al. Prevalence of elevated ALT values, HBsAg, and anti-HCV in the primary care setting and evaluation of guideline defined hepatitis risk scenarios. J Hepatol. 2015;62:1256–64. https://doi.org/10.1016/j.jhep.2015.01.011.
18. Rein DB, et al. The cost-effectiveness of birth-cohort screening for hepatitis C antibody in US primary care settings. Ann Intern Med. 2012;156:263–70. https://doi.org/10.7326/0003-4819-156-4-201202210-00378.
19. Liu S, Cipriano LE, Holodniy M, Goldhaber-Fiebert JD. Cost-effectiveness analysis of risk-factor guided and birth-cohort screening for chronic hepatitis C infection in the United States. PLoS One. 2013;8:e58975. https://doi.org/10.1371/journal.pone.0058975.
20. Isenhour CJ, Hariri SH, Hales CM, Vellozzi CJ. Hepatitis C antibody testing in a commercially insured population, 2005–2014. Am J Prev Med. 2017;52:625–31. https://doi.org/10.1016/j.amepre.2016.12.016.
21. Adebajo CO, Aronsohn A, Te HS, Reddy KG, Jensen DM, Reau N. Birth cohort HCV screening at is lower in the emergency department than the outpatient setting. Gastroenterology. 2015;148:S1101–2.
22. Konerman MA, Thomson M, Gray K, Moore M, Choxi H, Seif E, Lok ASF. Impact of an electronic health record alert in primary care on increasing hepatitis c screening and curative treatment for baby boomers. Hepatology. 2017;66:1805–13. https://doi.org/10.1002/hep.29362.
23. Patel RC, Vellozzi C, Smith BD. Results of hepatitis C birth-cohort testing and linkage to care in selected U.S. sites, 2012–2014. Public Health Rep. 2016;131(Suppl 2):12–9. https://doi.org/10.1177/00333549161310S203.
24. NY Pub Health L § 2171. Required offering of hepatitis C screening testing. 2014. https://law.justia.com/codes/new-york/2014/pbh/article-21/title-7/2171/.
25. Kim DD, Hutton DW, Raouf AA, Salama M, Hablas A, Seifeldin IA, Soliman AS. Cost-effectiveness model for hepatitis C screening and treatment: Implications for Egypt and other countries with high prevalence. Global Public Health. 2015;10:296–317. https://doi.org/10.1080/17441692.2014.984742.
26. Bruggmann P, et al. Birth cohort distribution and screening for viraemic hepatitis C virus infections in Switzerland. Swiss Med Weekly. 2015;145:w14221. https://doi.org/10.4414/smw.2015.14221.

27. Younossi Z, Blissett D, Blissett R, Henry L, Younossi Y, Beckerman R, Hunt S. In an era of highly effective treatment, hepatitis C screening of the United States general population should be considered. Liver Int. 2018;38:258–65. https://doi.org/10.1111/liv.13519.
28. Donnelly JP, Franco RA, Wang HE, Galbraith JW. Emergency department screening for hepatitis C virus: geographic reach and spatial clustering in central Alabama. Clin Infect Dis. 2016;62:613–6. https://doi.org/10.1093/cid/civ984.
29. Franco RA, et al. Characterizing failure to establish hepatitis C care of baby boomers diagnosed in the emergency department. Open Forum Infect Dis. 2016;3:ofw211. https://doi.org/10.1093/ofid/ofw211.
30. Galbraith JW, et al. Unrecognized chronic hepatitis C virus infection among baby boomers in the emergency department. Hepatology. 2015;61:776–82. https://doi.org/10.1002/hep.27410.
31. Hsieh YH, et al. Evaluation of the centers for disease control and prevention recommendations for hepatitis c virus testing in an urban emergency department. Clin Infect Dis. 2016;62:1059–65. https://doi.org/10.1093/cid/ciw074.
32. Zuure FR, Urbanus AT, Langendam MW, Helsper CW, van den Berg CH, Davidovich U, Prins M. Outcomes of hepatitis C screening programs targeted at risk groups hidden in the general population: a systematic review. BMC Public Health. 2014;14:66. https://doi.org/10.1186/1471-2458-14-66.
33. Bil JP, et al. Integrating hepatitis B, hepatitis C and HIV screening into tuberculosis entry screening for migrants in the Netherlands, 2013 to 2015. Euro Surveill. 2018;23:17-00491. https://doi.org/10.2807/1560-7917.ES.2018.23.11.17-00491.
34. Coyle C, Kwakwa H, Viner K. Integrating routine HCV testing in primary care: lessons learned from five federally qualified health centers in Philadelphia, Pennsylvania, 2012-2014. Public Health Rep. 2016;131(Suppl 2):65–73. https://doi.org/10.1177/00333549161310S211.
35. Tang W, et al. Diagnostic accuracy of tests to detect Hepatitis C antibody: a meta-analysis and review of the literature. BMC Infect Dis. 2017;17:695. https://doi.org/10.1186/s12879-017-2773-2.
36. Khuroo MS, Khuroo NS, Khuroo MS. Diagnostic accuracy of point-of-care tests for hepatitis C virus infection: a systematic review and meta-analysis. PLoS One. 2015;10:e0121450. https://doi.org/10.1371/journal.pone.0121450.
37. Shivkumar S, Peeling R, Jafari Y, Joseph L, Pant Pai N. Accuracy of rapid and point-of-care screening tests for hepatitis C: a systematic review and meta-analysis. Ann Intern Med. 2012;157:558–66. https://doi.org/10.7326/0003-4819-157-8-201210160-00006.
38. UNITAID. Hepatitis C, Diagnostics technology landscape. 2015.
39. Fourati S, Pawlotsky JM. Editorial: are additional tests needed to predict sustained virologic response in hepatitis C treated with interferon-free direct-acting antiviral combinations? Auth Reply Aliment Pharmacol Therap. 2018;47:849–50. https://doi.org/10.1111/apt.14520.
40. Levy MH, Larney S. The ethics of hepatitis C "treatment as prevention" among prisoners. Hepatology. 2015;61:402. https://doi.org/10.1002/hep.27195.
41. Montoy JC, Dow WH, Kaplan BC. Patient choice in opt-in, active choice, and opt-out HIV screening: randomized clinical trial. BMJ. 2016;532:h6895. https://doi.org/10.1136/bmj.h6895.

Hepatitis C Elimination and Advocacy Groups

10

Charles Gore

Advocacy for the elimination of hepatitis C needs to be seen within the context of advocacy for viral hepatitis as whole. That is what put elimination of hepatitis C on the global public health agenda and resulted in the commitment by 193 countries to eliminate hepatitis C by 2030; and advocacy for viral hepatitis at a global level really began at a meeting of patient representatives from around the world in Barcelona in 2007.

This is not to say that there was no hepatitis C advocacy before that. The contaminated blood scandals from the mid-1980s to mid-1990s in countries such as France, Canada, Ireland, Italy and Japan prompted public outrage. Thanks to organised and effective action, victims of contaminated blood products successfully sought compensation through national legal systems [1]. Haemophilia organisations, in particular, were key drivers of advocacy efforts, although in Ireland efforts were spearheaded by Positive Action representing women infected through Anti-D. Yet despite the success that resulted in justice for many thousands of victims, the scandals did little to draw attention to hepatitis C as a whole. Haemophilia organisations leading the advocacy efforts distanced themselves from other groups representing people living with HIV/AIDS and hepatitis C, presenting them as 'undeserving' victims and perpetuating stigma surrounding these infectious diseases [1]. Furthermore, despite rates of hepatitis C infection from contaminated blood surpassing that of HIV, hepatitis C is almost entirely omitted from reporting on the scandals.

Some countries also held hepatitis C awareness days. A number of European patient groups, who later coalesced into the European Liver Patients Association, held a common hepatitis C awareness day from 2003 and tried to create a world hepatitis awareness day, but it never achieved real traction outside parts of Europe.

C. Gore (✉)
Medicines Patent Pool, Geneva, Switzerland
e-mail: cgore@medicinespatentpool.org

A. Hatzakis (ed.), *Hepatitis C: Epidemiology, Prevention and Elimination*,
https://doi.org/10.1007/978-3-030-64649-3_10

The meeting in Barcelona in 2007 was an attempt to address this. Groups across the world were invited to choose a representative for their geographic region, and the meeting had representatives from Europe, South America, North America, Australasia, North Africa and Asia. Unfortunately there was no representation from sub-Saharan Africa. There was also a representative from the HIV department of the World Health Organization (WHO) regional office for Europe as an advisor. The group was asked two questions: did they want to start a truly global hepatitis day and did they want to have one just for hepatitis C or for viral hepatitis, meaning predominantly hepatitis B and C together.

At that time, viral hepatitis did not feature at all as a concept in global health. In fact, out of the 8000 people working for WHO whether in headquarters in Geneva or in regional or country offices, not a single employee had 'hepatitis' in their job title [2]. In as far as WHO was addressing viral hepatitis, it was centred entirely on the hepatitis B vaccine as part of the Expanded Program on Immunization. As a consequence, viral hepatitis was omitted from the Millennium Development Goals [3].

In this vacuum of awareness and leadership, the representatives of the affected community decided to create World Hepatitis Day. They also decided that it should encompass both hepatitis B and C on the grounds that the numbers of infected individuals worldwide and the annual death toll, estimated at the time to be 500 million people and a million deaths, respectively, were so huge they could not be ignored. Later that year, the World Hepatitis Alliance was set up in Geneva to run World Hepatitis Day.

The first World Hepatitis Day was held on May 19, 2008. The theme was *Am I Number 12?* to highlight that an estimated 1 in 12 of the global population was living with chronic viral hepatitis.

Photo: courtesy The Hepatitis C Trust

Photo: courtesy ALPC Egypt

Five months prior to the day, the World Hepatitis Alliance wrote to the WHO Director-General, Dr. Margaret Chan, asking three questions: Who was in charge of viral hepatitis at WHO? What were WHO's targets for the prevention and control of viral hepatitis? Would the Director-General attend the press conference in Geneva to launch World Hepatitis Day? There was no reply until 3 days before World Hepatitis Day when WHO said they would send someone to the press conference on the day. This was Dr. Craig Shapiro who had been seconded by the US Centers for Disease Control and Prevention (CDC) to WHO's Expanded Program on Immunization.

Although World Hepatitis Day achieved considerable traction worldwide, many patient advocacy groups reported that, despite their best efforts, they were unable to persuade their governments to participate. The reason, or perhaps more properly the excuse, given was that World Hepatitis Day was not an official day. At the time, there were only six official WHO days—World TB Day, World Malaria Day, World AIDS Day, World Health Day, World No Tobacco Day and World Blood Donor Day. There is also an official World Immunization Week.

The World Hepatitis Alliance decided that if making World Hepatitis Day an official day was what was needed to ensure governmental participation, then it would do exactly that. By then, Dr. Shapiro's term at WHO had ended, and he was replaced by another US CDC secondee, Dr. Steven Wiersma. Dr. Wiersma had set up the hepatitis prevention and control programme in Florida and was a strong proponent of increased visibility for viral hepatitis. Through his contacts in the WHO Director-General's office, he was able to advise the World Hepatitis Alliance on the steps needed to make World Hepatitis Day an official day, namely, persuade at least one WHO Member State to put hepatitis on the World Health Assembly agenda,

persuade a Member State to draft a resolution making it an official day and then persuade the other 192 Member States to adopt the resolution.

Through this process, the World Hepatitis Alliance changed from an organisation principally focused on awareness-raising into one centred on advocacy. At the same time, it formalised its structure to become what it is now—the global voice for people living with viral hepatitis, its board elected by its 250+ member patient organisations [4].

Initially there was much scepticism that it would be possible to persuade the world to agree to an official day, given the pervading *zeitgeist* of moving away from a disease-specific to a more general health system approach to global health. In addition, although there were only six official WHO days, there were already very, possibly too, many semi-official ones such as World Cancer Day. The World Hepatitis Alliance, however, believed that there was an extremely persuasive argument for a World Hepatitis Day and wrote to the ministers of health of all 193 WHO Member States, making the case that hepatitis was an exception and that nowhere else in health was there such a wide discrepancy between the huge burden and the very low levels of awareness and that, because these are infectious diseases, lack of awareness was leading to continuing transmission.

As a result of these letters, Afghanistan, Brazil, China and Oman put viral hepatitis, for the very first time, on the agenda of the WHO Executive Board meeting for January 2009. After that meeting, Brazil drafted a resolution to make World Hepatitis Day an official day for consideration at the World Health Assembly. However, the Brazilian Ministry of Health was resistant to input from the community into other clauses in the resolution. The World Hepatitis Alliance, in particular, felt that the resolution could have been improved and was a missed opportunity.

In the event, 2009 was the year that the threat of H1N1 flu became a major issue. The possibility of a major pandemic led the WHO Director-General to shorten the World Health Assembly by several days so that Ministers of Health could return home to be prepared. This meant that about half of the agenda items had to be postponed to the following year. Viral hepatitis was one of those items.

Although this was disappointing for advocates, it offered an opportunity to build a relationship with the Brazilian Ministry of Health. As a result, the community was able to work on a new draft of the resolution, which called for a range of actions from countries but also from WHO. While this was being drafted, the World Hepatitis Alliance commissioned on behalf of WHO the first ever survey of countries' policies around viral hepatitis. The ensuing report *Viral Hepatitis: Global Policy* was launched in April 2010, a month before the World Health Assembly. It demonstrated very clearly the need for global action to tackle viral hepatitis [5].

In May 2010, the World Health Assembly duly passed Resolution 63.18 and made World Hepatitis Day an official WHO day, although the date was changed to avoid a possible clash with the World Health Assembly from May to July 28th, the birthday of Baruch Blumberg, who discovered the hepatitis B virus. It also forced WHO to set up a unit, called the Global Hepatitis Programme, to oversee viral hepatitis across the world. For the first time, WHO now had staff dedicated to viral hepatitis.

This new visibility helped advocates nationally engage with their governments. However, few countries had a national plan either for viral hepatitis or specifically

for hepatitis C. Part of the reason was the treatment for hepatitis C, interferon and ribavirin. It was expensive and toxic and had limited efficacy. What advocacy there was tended to centre on persuading governments to broaden access to treatment and on persuading the pharmaceutical companies to lower prices, particularly in developing countries, for example, Ukraine and India.

Hepatitis B had always been a candidate for elimination because of the availability of a very cheap and very effective vaccine described in 1981 as the first anti-cancer vaccine [6]. Elimination of hepatitis C, however, had not been on the agenda because of the lack of a vaccine and the relative ineffectiveness and toxicity of the treatment. As new drugs that target the virus itself and prevent it replicating, known as direct-acting antivirals (DAAs), started showing spectacular results in trials, the elimination of hepatitis C, at least as a major public health concern, became a possibility. Unfortunately, governments were showing little appetite for action in the 3 years following the 2010 resolution.

The World Hepatitis Alliance decided that it needed to put together a core group of countries to lead the way, a tactic that had worked in the area of flu. The Alliance persuaded Brazil and Indonesia to co-host a side meeting during the 2013 World Health Assembly with this aim. The meeting featured, in addition to the co-hosts, speakers from the Mongolian, Scottish, Egyptian and Japanese delegations. They decided that rather than forming a group of champion countries, they would prefer to push for a new resolution. Egypt promptly submitted a request for it to be included in the 2014 agenda, and Brazil volunteered to draft the resolution.

Almost immediately after the Assembly, the head of the Brazilian Ministry of Health's Department of HIV and hepatitis was dismissed. The new head, who came from outside the department, had too much to do settling into the job and so asked the World Hepatitis Alliance to prepare the first draft. This gave advocates the opportunity to include a clause calling for the elimination of hepatitis B and C. WHO advised that this was too controversial, given the uncertainty over whether it was achievable. The clause ended up being watered down, and the resolution, when it was adopted in May 2014, requested the WHO Director-General 'to examine the feasibility of and strategies needed for the elimination of hepatitis B and hepatitis C with a view to potentially setting global targets' [7]. Critically, this put elimination centre-stage.

Furthermore, the resolution empowered WHO's Global Hepatitis Programme to commission modelling from University College London to see whether elimination was possible by 2030 to synchronise with the Sustainable Development Goals' timeline. Advocates attended a meeting with WHO at the end of 2014 to discuss the results of the modelling. Elimination in the technical sense of meaning no new cases in a defined region was considered impossible in the time frame, especially in the case of hepatitis C, where there is no vaccine available or even on the near horizon. So it was agreed that the goal would be 'elimination as a major public health concern'. Modelling suggested that a reduction of incidence of 90% overall could be achieved by 2030 and split into a 95% reduction in new cases of hepatitis B and a 80% reduction in new cases of hepatitis C, together with a reduction in mortality of 65% for both diseases. 2015 was chosen as the baseline year for these reductions.

To support this, targets for diagnosis and treatment were proposed. The most contentious of these were the diagnosis targets of 90% of those with hepatitis B and

C diagnosed by 2030. The treatment targets were set at 80% of those eligible to be treated by 2030, but these targets were dependent on the diagnosis targets since someone undiagnosed cannot, by definition, be eligible for treatment. These were then incorporated by WHO into a draft for the first Global Health Sector Strategy (GHSS) on viral hepatitis.

While the GHSS was in development, in light of its omission from the Millennium Development Goals, global advocates ran a campaign, writing to every Ministry of Health to have viral hepatitis officially acknowledged as a global health and development priority as part of the United Nations Sustainable Development Goals (SDGs). As a result in Goal 3.3, world leaders pledged to 'combat hepatitis' [8].

Ahead of the consideration of the GHSS by Member States, in September 2015, at the inaugural World Hepatitis Summit, over 500 policymakers, patient groups and physicians showed their support for the strategy by issuing the Glasgow Declaration, which affirmed the belief that elimination is possible and urged governments to work with WHO towards global targets for prevention, diagnosis and treatment [9].

During 2015, WHO held a series of consultations with Member States on the draft GHSS, and advocates were also asked for input. There seemed to be a broad consensus that this was an excellent strategy and the goal of elimination was strongly supported. Based on feedback, the strategy went through various drafts. In one of these, the target of 90% diagnosis was dropped. With 325 million people living with hepatitis B or C and perhaps only a small fraction of them diagnosed [10], diagnosing around 300 million people was always going to be the major challenge and therefore the target most likely to be contested by Member States. At once, advocates from the World Hepatitis Alliance appealed to WHO to reinstate the diagnosis target, arguing that the treatment targets lost all meaning without it. Happily, of all the WHO departments, the HIV and Hepatitis Department is the most experienced in relation to advocacy groups and the most willing to listen to them. The diagnosis target was reinstated.

While the consultations were happening, the WHO Western Pacific Regional Office developed a regional plan based on the GHSS with essentially the same targets [11]. This was debated at the Western Pacific Regional Committee Meeting in Guam in September 2015. During the discussion, out of the blue, China proposed removing all the specific numbers in the targets, leaving them little more than vague aspirations. If this had been agreed, it would have led to the removal of precise targets in the GHSS, effectively emasculating it. This seemed to vindicate WHO's concerns about countries like China with huge burdens being unwilling to commit to action on the enormous scale necessary.

It so happened that advocates from the World Hepatitis Alliance were present at the meeting and, because the World Hepatitis Alliance is in 'official relations' with WHO, were permitted to intervene. They reminded China that the extraordinary progress in reducing mother-to-child transmission of hepatitis B in the region, and particularly in China itself, was the result of having a clear target of achieving prevalence in children of less than 2% by 2012 and then 1% by 2017 [12]. In other words, defined targets produce results. China relented and withdrew its objection.

By the time of the World Health Assembly in May 2016 and more than a year of consultations with countries in all six regions of the world, it looked as though the

GHSS and its goal of the elimination of hepatitis B and hepatitis C would be adopted by all 193 Member States. However, at the last minute, Russia suggested to the World Hepatitis Alliance that it wanted to make very significant changes to the wording of the GHSS, albeit without altering its goal or its targets. This posed a huge risk because, once countries began discussing the detail of the GHSS again, anything could happen, including changes to the targets, even a failure to agree and the postponement of its adoption. Happily, the World Hepatitis Alliance had very good relations with the Russian delegation and was able to persuade them that getting the first global strategy on viral hepatitis adopted was too important to jeopardise at such a late stage. The GHSS was duly adopted, and 193 countries have agreed to eliminate hepatitis C by 2030 [13].

This global agreement on the goal of elimination provided a major tool for advocates, but even before that, hepatitis C advocacy had been growing in strength because of the arrival of new treatments that are far more effective and far more tolerable than interferon. Initially, these direct-acting antiviral (DAA) drugs appeared at very high prices. Because they can cure almost 100% of those who take them, they were cost-effective, but the big issue became—were they affordable? With so many people infected with hepatitis C—around 70 million worldwide [8]—governments baulked at making such expensive drugs available to everyone, and many introduced rationing, typically limiting access to those with advanced liver disease. Advocacy focused on fighting the restrictions on access. Different approaches were taken in different countries:

- **Direct action to gain media coverage:** From 2014, the Spanish group Plataforma de Afectados por Hepatitis C carried out hospital lock-ins, hunger strikes and demonstrations demanding access to treatment for all. The group, made up of thousands of people living with hepatitis C, and their actions gained widespread national and international media coverage. This coverage focused attention on the lack of access to these life-saving drugs and forced the government to act, with the Government of Spain guaranteeing new treatment regimens to all those diagnosed with hepatitis C in Spain in 2015.
- **Negotiations to improve access to treatment:** In Ukraine, advocacy efforts from the Alliance for Public Health resulted in greater access to hepatitis C diagnostics and treatment. Through its advocacy, the organisation was able to purchase new direct-acting antiviral drugs at a price of $900 per treatment course, a price significantly lower than the original cost and more than ten times lower than the cost in other middle-income countries. Subsequently, this set a precedent for new pricing strategies and helped facilitate Ministry of Health negotiations with the drug manufacturer and enabled the Ministry to purchase treatment for the government programme at the Alliance's price. Such activities also resulted in updates to the national protocol for hepatitis C treatment and the inclusion of direct-acting antivirals.
- **Championing the cause from the inside:** After years of unsuccessfully treating hepatitis C patients, Ricardo Baptista Leite, medical doctor and member of the Portuguese parliament, saw the development of direct-acting antivirals as an opportunity to change the lives of people living with hepatitis C but lamented

the high treatment prices and general lack of awareness. To tackle this issue, in 2014 he developed a new methodology for action—his 'Consensus Method'—and published the book 'Strategic Consensus for the Integrated Management of Hepatitis C in Portugal'. This was a ground-breaking study, summarising 6 months of discussions among Portugal's leading experts in the field. Given Dr. Leite's unique position within parliament, he was able to increase the profile of the disease enormously, and the study assisted the government in developing a sustainable programme to treat all patients.

- **Sustained and varied advocacy activities:** Patient organisation Grupo Otimismo achieved results in Brazil through sustained but varied approaches to advocacy. In July 2015, the organisation showed the power of sheer numbers by delivering a petition with over 65,000 signatures to government and the pharmaceutical companies, calling for a fair price for treatment. This was followed by media activity with the organisation delivering a 240 m long flag signed by patients to the Ministry of Health. This produced immediate results with the Ministry agreeing to work with civil society to accelerate access to new treatments. Just 4 months later, DAAs were incorporated into the Brazilian treatment schedule.

Since their introduction, prices for the new drugs have fallen greatly, aided by competition in the developed world. A number of pharmaceutical companies have drugs for hepatitis C, allowing governments to play off one against another. The pharmaceutical companies have therefore been willing to negotiate prices at which the drugs are not just cost-effective but actually cost-saving, especially in return for a commitment to treat very large numbers or a deal aimed at elimination. One of the first of these deals was in Australia where the government committed AUS $1 billion over 5 years for what is effectively an unlimited amount of the drugs [14].

In developing countries, Gilead licensed their drugs to generic manufacturers, and BMS and Pharco have licensed drugs through the Medicines Patent Pool [15]. This has brought prices for a full course of treatment to below US $200, and there is an expectation this will fall to US $50.

Affordable drugs are only one part of the story. Much else needs to happen to achieve elimination. Although many more countries now have national strategies for hepatitis C, very few have elimination plans. This is important because it is becoming increasingly clear that what is going to derail elimination is not access to the drugs but a failure to diagnose enough people and then link them into care. A perfect example is Australia where the huge numbers treated and cured in the first year of their deal with the pharmaceutical companies put them well on track for elimination but where treatment numbers have since fallen significantly. Although Australia has theoretically diagnosed 85% of people living with hepatitis C, there has turned out to be a huge difference between 'diagnosed' and 'ready to be treated' with perhaps the majority having fallen out of the patient pathway. This could mean that globally, where only 20% are estimated to have been diagnosed, the real situation is much more challenging.

Advocacy will now have to turn its attention to increasing diagnosis and linkage to care. A good example in the developed world is England where the national

hepatitis C charity, The Hepatitis C Trust, joined up with an HIV charity, the National AIDS Trust, and campaigned successfully to persuade the government to introduce opt-out testing for blood-borne viruses for all new receptions in all prisons. In the developing world, the cost of diagnostics has become a major issue. In some countries, the cost of the diagnostics is now more than the cost of treatment. This is not a traditional area for advocates, and experience and knowledge will have to be built to make advocacy effective.

Graphic: courtesy World Health Organization

Equally, advocacy will be necessary to hold governments to account all along the road to elimination by 2030. The World Health Organization has produced a monitoring and evaluation framework against which governments will be expected to report. The first major report will be in May 2021 at the World Health Assembly where progress against the 2020 interim targets will be assessed. These interim targets are a little more challenging in some regions than others as a result of the regional action plans that each WHO region has developed and agreed with the Member States from those regions. So, whereas the diagnosis target is 30% of those with hepatitis C [10], in Europe Member States have agreed to diagnose 50% of those with hepatitis C and 75% of those with cirrhosis or liver cancer caused by hepatitis C by 2020 [16]. These targets will provide advocates with real numbers against which to judge their government's progress.

References

1. Asia Catalyst. AIDS blood scandals: what china can learn from the world's mistakes. 2007. http://www.asiacatalyst.org/wp-content/uploads/2014/09/AIDS_blood_scandals_rpt_0907.pdf. Accessed 29 Apr 2018.
2. Gore C. Calling for an end to the neglect of viral hepatitis. The Guardian. https://www.theguardian.com/global-development-professionals-network/2013/aug/09/viral-hepatitis-disease-cancer-mdgs. Accessed 29 Apr 2018.
3. Adriano J. The case for keeping viral hepatitis in the SDGs. SciDevNet. 15 April 2015. http://www.scidev.net/global/disease/editorials/health-viral-hepatitis-sdgs.html. Accessed 29 Apr 2018.
4. World Hepatitis Alliance. Who we are. www.worldhepatitisalliance.org. Accessed 29 Apr 2018.
5. Viral Hepatitis: Global Policy. http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&gid=18148&Itemid=270&lang=en. Accessed 29 Apr 2018.
6. Centers for Disease Control and Prevention. The Pink Book: Hepatitis B. https://www.cdc.gov/vaccines/pubs/pinkbook/hepb.html. Accessed 29 Apr 2018.
7. World Health Assembly Resolution WHA67.6. http://apps.who.int/gb/ebwha/pdf_files/WHA67-REC1/A67_2014_REC1-en.pdf#page=1. Accessed 29 Apr 2018.
8. World Hepatitis Alliance. Our story. http://www.worldhepatitisalliance.org/about/our-story. Accessed 29 Apr 2018.
9. World Hepatitis Alliance. Building Momentum, Making the Case. http://www.who.int/hepatitis/publications/whs-2015-report.pdf?ua=1. Accessed 29 Apr 2018.
10. WHO Global Hepatitis Report. http://apps.who.int/iris/bitstream/handle/10665/255016/9789241565455-eng.pdf?sequence=1&isAllowed=y. Accessed 29 Apr 2018.
11. WHO Regional Action Plan for Viral Hepatitis in the Western Pacific 2016–2020. http://www.wpro.who.int/hepatitis/resource/features/regional_action_plan/en/. Accessed 29 Apr 2018.
12. WHO WPRO Hepatitis B Fact Sheet. http://www.wpro.who.int/mediacentre/factsheets/fs_20120219_hepb/en/. Accessed 29 Apr 2018.
13. World Health Assembly Resolution WHA69.22. http://apps.who.int/gb/ebwha/pdf_files/WHA69-REC1/A69_2016_REC1-en.pdf#page=1. Accessed 29 Apr 2018.

14. Australia leads the world in hepatitis C treatment—what's behind the story? http://theconversation.com/australia-leads-the-world-in-hepatitis-c-treatment-whats-behind-its-success-81760. Accessed 29 Apr 2018.
15. The Medicines Patent Pool. www.medicinespatantpool.org. Accessed 29 Apr 2018.
16. Action Plan for the health sector response to viral hepatitis in the WHO European Region. http://www.euro.who.int/__data/assets/pdf_file/0017/318320/European-action-plan-HS-viral-hepatitis.pdf?ua=1.

HCV Elimination in Australia

11

Behzad Hajarizadeh, Marianne Martinello, and Gregory J. Dore

11.1 Introduction

The epidemic of hepatitis C virus (HCV) infection in Australia has been primarily driven by injecting drug use. HCV incidence was high in the 1980s and 1990s, followed by a decline in early- to mid-2000s, initially related to reduced heroin supply [1]. Ongoing HCV incidence of several 1000 infections/year over the last decade has been despite high-coverage harm-reduction services for people who inject drugs (PWID) [2].

Despite some control of HCV spread, and availability of subsidised interferon-containing treatment since 1997 [3], the burden of HCV-related liver disease continued to increase [3, 4], given an "ageing cohort" of the HCV infected population, low HCV treatment uptake (1–2% per annum [4]), and sub-optimal efficacy of interferon-containing therapy. The rising HCV burden was demonstrated through increases in advanced liver disease complications including hepatocellular carcinoma (HCC) [5, 6], HCV-related liver transplants [3], and liver-related mortality [7].

In 2015, an estimated 188,700 individuals were living with chronic HCV infection in Australia [2], of whom ~80% had been diagnosed and 24% had ever received interferon-containing therapy [3, 8]. Interferon-free direct-acting antiviral (DAA) therapy was only available through clinical trials and pharmaceutical company compassionate access programs (from late 2014), and generic importation (from mid-2015), prior to Australian Government-funded unrestricted access in March 2016 [9].

B. Hajarizadeh · M. Martinello · G. J. Dore (✉)
Viral Hepatitis Clinical Research Program, The Kirby Institute, UNSW Sydney, Sydney, NSW, Australia
e-mail: bhajarizadeh@kirby.unsw.edu.au; mmartinello@kirby.unsw.edu.au; gdore@kirby.unsw.edu.au

A. Hatzakis (ed.), *Hepatitis C: Epidemiology, Prevention and Elimination*,
https://doi.org/10.1007/978-3-030-64649-3_11

11.2 History of Response to Hepatitis C in Australia: Paving the Path Towards HCV Elimination

Although the launch of the government-funded unrestricted DAA access program has been considered as the major milestone in Australia's response to HCV, there is a two-decade history of a national HCV response that provided the foundation for this development.

One of the key factors, driving the successful HCV response in Australia, is the development of national HCV strategies, with the first *National Hepatitis C Strategy* launched in 2000, while the fifth Strategy launched in 2018 [10]. National HCV strategies, developed with contributions of all key stakeholder groups, including government, drug user and hepatitis community organisations, and medical and academic communities, established a comprehensive framework to guide actions in national and state level. Partnerships between these stakeholder groups continued in development of the *Australian recommendations for the management of hepatitis C infection: a consensus statement 2016*, released for the DAA program launch in March 2016 (last update on June 2020) [11].

National HCV strategies have considered primary care and addiction medicine physicians as key groups in diagnosis and clinical management of HCV infection. Major HCV education initiatives for these groups commenced in the early 2000s, together with pilot HCV therapy prescribing projects [12]. It has facilitated the high levels of HCV screening [13] and established the foundation for development of a broad prescriber base in Australia's DAA program in 2016 with all registered medical practitioners able to prescribe DAA therapy [9, 14].

Harm reductions strategies have been implemented broadly for PWID since the early 1990s. It has placed Australia among only four countries with high coverage of both needle and syringe programmes (NSP), and opioid substitution therapy (OST) in 2017 (defined as >200 needle-syringes distributed per PWID and >40 OST recipients/100 PWID), with the other three countries being Austria, the Netherlands, and Norway [15]. High-coverage harm-reduction services in Australia maintained low HIV prevalence of 1% among PWID [16] and provided an access point for PWID to engage in HCV testing, thereby facilitating a high level of HCV screening (~90%) [13, 17].

Australia's unrestricted DAA treatment program was developed through strong advocacy, robust epidemiological data, bipartisan political support, and established partnerships between all stakeholders. In 2014, the Pharmaceutical Benefits Advisory Committee (PBAC), an independent body that evaluates new therapeutic applications for the Australian Government subsidisation, organised a meeting to discuss subsidising DAA therapy with all key stakeholder groups present, including the pharmaceutical industry. The hepatitis and drug user community-based organisation representatives were particularly vocal in advocating "access to all", rather than a restricted access strategy that most high-income countries had pursued [18, 19].

After several months of price negotiations between the Australian Government and the pharmaceutical companies, in December 2015, the government announced

the allocation of $AUS one billion ($US 800 million) over the 2016–2020 period with a cap on expenditure (probably $AUS 250–300 million per annum) but with no cap on the number of individuals treated. It means that the higher number of patients treated annually (assuming the cap is reached each year), the lower the overall price per patient course. Other key features of Australia's DAA treatment program include no restrictions based on liver disease stage or drug and alcohol use, broad prescriber base with all registered medical practitioners able to prescribe DAA treatment, retreatment (including for reinfections) allowed, and minimal out-of-pocket cost for the patients with a co-payment of $AUS 7–36/month [9, 14].

The initial DAA regimens were subsidised from March 2016 (sofosbuvir/ledipasvir, sofosbuvir plus daclatasvir, and sofosbuvir plus ribavirin), with additional regimens subsidised in May 2016 (paritaprevir/ritonavir/ombitasvir plus dasabuvir with or without ribavirin), in January 2017 (grazoprevir/elbasvir), and in August 2017 the first pangenotypic regimen (sofosbuvir/velpatasvir). A further two pangenotypic DAA regimens (glecaprevir/pibrentasvir and sofosbuvir/velpatasvir/voxilaprevir) were included in August 2018 and April 2019, respectively. Importantly, with inclusion of all major DAA regimens, clinicians are free to choose individual patient regimens without consideration of pricing/cost.

11.3 Initial DAA Uptake

By the end of December 2017, an estimated 58,480 individuals, equating to 31% of the total individuals living with HCV in Australia, received DAA treatment [14]. It includes 4340 individuals receiving treatment in 2014 and 2015, prior to the government-funded DAA program, through clinical trials, pharmaceutical company compassionate access programs, and generic importation [9], and 54,140 individuals receiving treatment from March 2016 to December 2017, during the first 22 months of the DAA program. In contrast, over the preceding two decades (1997–2015), only 46,310 individuals received interferon-containing therapy [9].

The number of individuals initiating DAA treatment was highest during the initial 6 months of the DAA program, consistent with a "warehouse" effect with a large number of patients in specialist clinics awaiting DAA therapy access (Fig. 11.1). Subsequent declines in DAA initiations were followed by a relatively stable trend in treatment uptake during December 2016 to September 2017, with a monthly treatment initiation number of 1700–2250 (Fig. 11.1). Preliminary data indicates a further decline in DAA treatment numbers in late 2017 and early 2018. However, a modelling study demonstrated that assuming scenarios of monthly treatment initiations of 1100–1900 in 2018, and 1100–1500 in 2019 onwards, Australia will meet the World Health Organization (WHO) elimination targets [20] by 2025–2030 [21].

To control HCV-related mortality and disease burden, treatment of patients with advanced liver disease is a priority. In Australia, it is estimated that 70% of the total population with HCV-related cirrhosis (including those who remain undiagnosed) initiated DAA treatment from 2014 to 2017 [9].

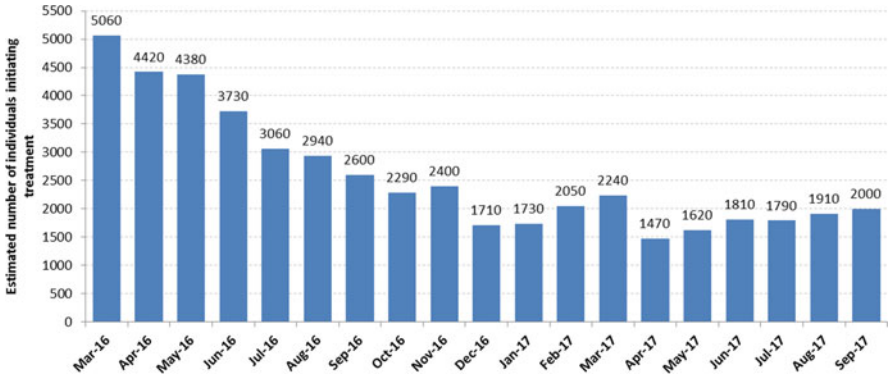


Fig. 11.1 Estimated monthly number of individuals initiating direct-acting antiviral treatment in Australia between March 2016 and September 2017

One of the key features of the Australian DAA program, enabling rapid treatment scale-up, is having no restrictions based on the liver disease stage or drug and alcohol use. It has broadened DAA therapy across the HCV-infected population, including marginalised populations at greater risk of HCV transmission (i.e. PWID, people living with HIV, and prisoners). The annual Australian Needle and Syringe Program Survey (ANSPS) is a cross-sectional survey, enrolling around 2500 PWID from 50 NSP sites each year across Australia. This survey indicated that that 41% of participants with HCV infection self-reported DAA initiation in 2017 (compared with 10% in 2015) [22, 23]. Among individuals with HIV/HCV coinfection (predominantly men who have sex with men), more than 40% have initiated DAA therapy in the first year of DAA program [24].

Prison-based access to DAA therapy is another important feature of the Australian DAA program. An estimated 1500 prisoners received DAA treatment during the first year of DAA program (March 2016 to February 2017), with an expected upward trend in 2017 and 2018.

11.4 Diversity of Models of Care and Ease of DAA Access

A key to Australia's success to date in DAA therapy uptake has been the development of a range of models of HCV care for different populations in different settings, and the involvement of a broad DAA prescriber base including general practitioners (GPs) and other non-specialist clinicians. During the first 16 months of the DAA program (March 2016 to June 2017), the proportion of patients initiated on DAA therapy by specialists (predominantly gastroenterologists and infectious disease physicians) decreased from 78% to 39% (Fig. 11.2) [9, 14], a trend which indicates the broadening of models of care.

There is also limited administrative requirement for DAA approval, with generally a short (1–2 min) phone call for authorisation, with brief details provided

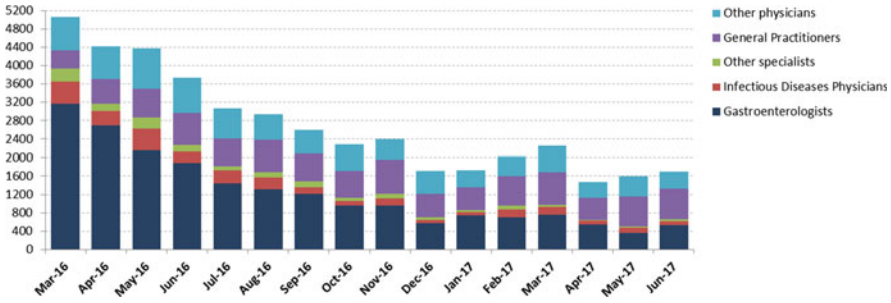


Fig. 11.2 Estimated monthly number of individuals initiating direct-acting antiviral therapy in Australia between March 2016 and June 2017, by prescriber type

including name, universal healthcare (Medicare) number, HCV genotype (not required if using pan-genotypic regimens), presence of cirrhosis or not, and DAA regimen and duration. Once approval is provided, DAA dispensing can occur the same day. The large majority (around 80%) of DAA therapy is now dispensed through community (retail) pharmacies, further enhancing the suitability for patients, many of whom would have travelled large distances to access therapy in hospital-based pharmacies which was the norm in the interferon-based HCV treatment era.

Some examples of the diversity of Australian HCV care delivery models and strategies are described as the following cases studies.

11.4.1 Case Study 1: Kirketon Road Centre

The Kirketon Road Centre (KRC) is an integrated model of care in a primary healthcare facility, providing a comprehensive range of care, including general medical care, clinical care of HIV, viral hepatitis and sexually transmissible infections, harm reduction services (e.g. OST and NSP), women's health services (e.g. Pap smears, contraception, and pregnancy testing and advice), mental health services, counselling and psychosocial services, nursing and peer-support, and social care (e.g. housing, social security, and welfare information and assistance) to marginalised populations, including at-risk youth, PWID, and sex workers [25, 26].

Established in 1987, KRC is located in Kings Cross, known as the "red-light" district of Sydney, with a long history of drug trade and sex work [26]. Encompassing the principles of acceptability, accessibility, and affordability of healthcare provision, KRC provides free of charge and anonymous services, both in-house and through a clinical outreach program, including joint outreach with peer organisations for PWID and sex workers. The majority of clinical care services, including HCV assessment and treatment, are provided by non-specialists, with an infectious diseases physician visiting KRC on a monthly basis for management of complex patients.

Since availability of government-funded DAAs in Australia in March 2016, KRC has provided DAA treatment, both in-house and at outreach clinics. Individualised

DAA dosing options are utilised, including daily supervised and weekly dosing, following an assessment of adherence support needs [27]. A study of 72 PWID initiating DAA therapy in KRC showed a successful outcome, with 96% ($n = 69$) of participants completing their planned treatment course and 82% ($n = 59$) achieving SVR in an intention-to-treat analysis. Among those completing treatment with no SVR ($n = 10$), all were lost to follow-up after treatment completion, with six of them having an undetectable HCV RNA at the end of treatment [27].

11.4.2 Case Study 2: Nurse-Led Model of Care in the Prison Setting

HCV prevalence is high in the prison setting in most countries [28], including in Australia (22% anti-HCV Ab positive) [29], given high rates of incarceration of PWID. Although drug law reform is needed to reduce these high rates, the prison setting does provide a good opportunity to engage traditionally hard-to-access populations in HCV care. Despite this opportunity, several challenges to DAA treatment in prisons remain, including prevalent comorbidities (e.g. mental health disorders and ongoing drug use), the low priority of healthcare for many prisoners, high transitioning rates between prisons and from prison to communities, complex organisational systems, and logistics (e.g. limited space and inadequate health professionals in many prisons).

In 2009, a novel nurse-led model of HCV care was developed and implemented in New South Wales prisons to enhance HCV treatment capacity via decentralised care with specialist input provided by telemedicine. This model involved substantial task transfer from specialist physicians to trained nurses who screened prisoners for HCV infection and conducted pre-treatment clinical assessments of those diagnosed with HCV infection, including a targeted medical history, physical examination, mental health assessment, supplementary laboratory tests, and transient elastography for liver disease staging. Further, the nurse triaged each patient in relation to comorbidities, and potential issues related to treatment adherence, classifying the patient into one of three categories: "A: suitable for treatment after discussion between the specialist physician and nurse only; B: suitable for treatment, but a teleconference with the specialist physician required; or C: needing face-to-face assessment by the specialist physician before the decision to treat could be resolved" [30]. The nurse initiated interferon-based HCV treatment for the patient and conducted all on-treatment and post-treatment clinical follow-up with seeking specialist's input via teleconference if required [30].

One study, evaluating the safety and effectiveness of a this nurse-led model of HCV care, using interferon-based therapy, in three prisons in New South Wales, demonstrated a treatment uptake, treatment outcome, and adverse events, consistent with those in community settings and tertiary clinics [30].

The successful model of nurse-led interferon-based HCV treatment in New South Wales prisons has been expanded in the DAA treatment era. The enhanced tolerability and ease of dosing has led to even fewer prisons requiring specialist review through telemedicine or face-to-face consultation, with the vast majority

commencing therapy following brief discussion or chart review via the nurse. Overall HCV treatment numbers have increased several-fold, with around 1000 prisoners treated in New South Wales prisons in 2017 (personal communication, Prof Andrew Lloyd), and the New South Wales nurse-led model of HCV care is being replicated in other Australian jurisdictions.

There are also successful experiences of community-based nurse-led HCV care [31, 32], indicating the potentials of this model for HCV care delivery in community, particularly in the regional areas. *Eliminating Hepatitis C Transmission by Enhancing Care and Treatment among HIV co-infected Individuals (co-EC)* is a project implementing a nurse-led model of HCV care in primary care settings in Victoria, to increase DAA treatment uptake in individuals co-infected with HIV [33].

Authorised nurse practitioners, experienced in HCV clinical management, can now prescribe DAAs independently, although the number of prescribers is small.

11.4.3 Case Study 3: ETHOS

Enhancing Treatment for Hepatitis C in Opioid Substitution Settings (ETHOS) was a project assessing the feasibility and effectiveness of a model of HCV care for PWID, integrating HCV clinical services into existing infrastructures providing drug treatment services to PWID [34]. The design of ETHOS was primarily based on evidence indicating that an already established engagement of PWID to drug treatment services could facilitate access to HCV care, given the convenient, familiar, and trusting environment, and reduced travel time and cost for clients [35].

Conducted between 2009 and 2014 in interferon-based treatment era, ETHOS provided on-site HCV assessment and treatment and peer support in OST clinics and community health centres in urban, regional, and rural areas. At enrolment, participants were assessed for HCV infection by a nurse or GP. Those with HCV infection were referred to an HCV specialist (infectious disease physician, hepatologist, or a GP with HCV training and prescribing rights) for further assessment and treatment, with HCV specialist services provided on-site at some centres [34]. Of 415 participants enrolled, 101 participants initiated interferon-based treatment, among whom 86% ($n = 87$) had $\geq$80% adherence to treatment and 74% ($n = 75$) achieved SVR [34].

Following availability of DAA in Australia, two projects in New South Wales have been designed using the same principles of integrating HCV care in drug treatment services. In *ETHOS Engage*, the same model of care as in ETHOS will be implemented with an addition of two interventions to further increase linkage to care and treatment uptake, including point-of-care HCV testing and on-site transient elastography to assess liver fibrosis [36]. *TEst and treat hepatitis C aMong needle and syringe PrOgram clients (TEMPO),* an in-progress project, will evaluate DAA therapy delivery through NSP sites, with peer-based support (ClinicalTrials.gov Identifier: NCT03492112).

11.4.4 Case Study 4: Kombi Clinic

Kombi Clinic is an innovative project in Queensland, providing outreach HCV clinical care services in a "one-stop shop" basis (https://www.kombiclinic.com/). Kombi Clinic outreach team includes GPs, nurses, laboratory technicians, and dieticians, providing HCV care services in a Kombi Volkswagen, including blood testing, transient elastography, and DAA treatment. This project particularly targets hard-to-access marginalised people such as those in hostels and homeless shelters who are less likely to get to mainstream medical facilities.

11.5 Insights from Mathematical Modelling Studies

The WHO targets for *Global HCV elimination as a public health threat* by 2030 (i.e. 90% of individuals with HCV infection diagnosed, 80% of eligible treated, 80% reduction in HCV incidence, and 65% reduction in HCV-related mortality [20]) are extremely ambitious, given that in 2017 only nine countries, including Australia, were shown to be "on-track" for HCV elimination [37].

A recent modelling study projected the progress of Australia towards achieving the WHO HCV elimination targets, assuming different treatment uptake scenarios [21]. This study indicated that even with a pessimistic scenario of a sharp decline in treatment uptake from around 21,500 individuals in 2017 to 13,950 in 2018 onwards, Australia would meet WHO targets of 80% reduction in HCV incidence by 2027 and 80% of patients treated by 2029. However, achieving the target of 65% reduction in HCV-related mortality would be challenging given an ongoing risk (albeit reduced) of advanced liver disease complications, including cirrhosis and HCC in those cured. It was projected that although the mortality reduction target among HCV viraemic population would be met in 2019–2027 (in different treatment uptake scenarios), in combined HCV viraemic and cured individuals, this target would not be met earlier than 2041 [21].

Another modelling study, focussing on DAA treatment uptake scenarios among PWID, indicated that a minimum of 4700 PWID treated/year (59/1000 PWID/year) for the next 15 years were required to meet the WHO target of 80% reduction in HCV incidence by 2030 [38]. Data from the annual Australian Needle and Syringe Program Survey (ANSPS), which enrols 2000–2500 PWID at 50 NSP sites each year, showed that 24% and 41% of participants with HCV infection self-reported DAA initiation in 2016, and 2017, respectively [22, 23]. Estimates of the current PWID population in Australia range from 68,000 to 118,000 [39], and with around 45% HCV viraemic, the number living with HCV is 30,000–53,000. Assuming the ANSPS is representative, 24% DAA uptake in 2016 would translate to 7,200–12,700, which is well above the treatment uptake in the first year of DAA therapy required to meet the incidence reduction criteria.

Another modelling study, investigating the impact of non-treatment based interventions, indicated that replacing anti-HCV Ab testing with point-of-care

HCV RNA testing for screening would save AUS$62 million and would gain 11,000 quality-adjusted life years [40].

11.6 Empirical Evidence for HCV Treatment as Prevention

Although mathematical modelling studies demonstrate the feasibility of HCV treatment as prevention, there are limited empirical studies evaluating the impact of HCV treatment scale-up in preventing HCV transmission. In Australia, several HCV treatments as prevention projects are in progress in key at-risk populations, including prisoners, community-based PWID, HIV/HCV co-infected individuals, and Indigenous Australians.

The Surveillance and Treatment of Prisoners with hepatitis C (SToP-C) study (ClinicalTrials.gov Identifier: NCT02064049) evaluated the feasibility and effectiveness of an HCV treatment as prevention strategy within four prisons in New South Wales (two maximum-security and two medium-security, including one female prison). SToP-C consisted of two major components: *surveillance and monitoring* phase, in which HCV status and risk behaviour evaluated at study entry with subsequent monitoring of HCV incidence and risk behaviour every 3–6 months for the total study duration, and *treatment scale-up* phase, in which all participants with detectable HCV RNA received 12 weeks sofosbuvir/velpatasvir therapy. HCV incidence within the combined four prison cohort was compared between the pre-treatment, and post-treatment scale-up periods. SToP-C was a 5-year project, with the enrolment commencing in October 2014 and the treatment scale-up phase commencing in the second half of 2017 across the four prisons. A total of 3691 prisoners were enrolled, including 719 individuals with active HCV infection (detected HCV RNA). HCV incidence before treatment scale-up was 8.3/100 person-years, declined to 4.4/100 person-years following treatment scale-up [41].

The *Hepatitis C Treatment and Prevention (TAP)* study (ClinicalTrials.gov Identifier: NCT02363517) is evaluating the feasibility and effectiveness of a network-based treatment approach ("bring a friend" strategy) on HCV incidence and reinfection among community-based PWID in Victoria [42]. TAP recruited primary and secondary participants, with primary participants being current PWID with chronic HCV ($n = 120$) and secondary participants being the injecting partners of the primary participants, identified using the "bring your friend" strategy ($n = 300$, HCV-infected and uninfected). Participants are randomised to three treatment groups. Group A receive supportive care, including counselling, and provision of injecting equipment. In Group B, primary participants receive DAA therapy, and secondary participants receive supportive care. In group C, primary and secondary participants with chronic HCV receive DAA therapy. Participants will be followed to evaluate the incidence of HCV primary infection and reinfection.

The Control and Elimination within AuStralia of hEpatitis C in people living with HIV (CEASE) study (ClinicalTrials.gov Identifier: NCT02102451) evaluated the feasibility of rapid scale-up of DAA therapy and population-level impact among

individuals with HIV/HCV co-infection in New South Wales [43]. The feasibility of HCV treatment as prevention in this population in Australia is enhanced given a relatively small HIV/HCV co-infected population (estimated 1900–2700 individuals, prominently men who have sex with men [24]) and favourable HIV care cascade (90% diagnosed and 96% of those diagnosed retained in HIV care [2]). Among 402 enrolled participants, annual DAA treatment uptake increased from 7% and 11% in 2014 and 2015, respectively, to 80% in 2016, with resultant reduction in estimated HCV RNA prevalence from 82% in 2014 to 8% in 2018, a clear demonstration of a reducing viraemic infection within the HIV population in Australia. Among those treated in 2016 and 2017, SVR12 was 96%, and 97%, respectively [43].

Eliminating Hepatitis C Transmission by Enhancing Care and Treatment Among HIV co-infected Individuals (Co-EC) study (ClinicalTrials.gov Identifier: NCT02786758) was another project among individuals with HIV/HCV co-infection, aiming to enhance HCV care in this population through a nurse-led model of care in primary care and tertiary care settings in Victoria. Co-EC was an observational study, assessing HCV treatment uptake and treatment response among individuals with HIV/HCV co-infection. This study was also assessing the impact of HCV treatment in HIV/HCV co-infected individuals on primary HCV and reinfection incidence and HCV prevalence in gay and bisexual men in Victoria. Among 186 participants initiaing treatment, SVR was 98% in both primary care and tertiary care settings [33].

Strategies for hepatitis C testing and treatment in Aboriginal communities that Lead to Elimination (SCALE-C) study will evaluate a community-based model of care providing point-of-care HCV testing, on-site transient elastography, and DAA therapy in Aboriginal Community Controlled Health Services (ACCHS) in New South Wales and South Australia. HCV infection disproportionately impacts Indigenous Australians (i.e. Aboriginal and Torres Strait Islander). Over the last 5 years, an extremely concerning 43% increase in newly diagnosed HCV has been documented in Indigenous Australians, compared with a 10% decrease in general Australian population [44]. SCALE-C has been designed to respond to the needs for specific models of care to provide equitable DAA access to Indigenous Australians. SCALE-C is a 3-year project recruiting participants from ACCHS and affiliated drug and alcohol services. This study will evaluate DAA treatment uptake and response to DAA therapy, including post-treatment reinfection, among participants with HCV infection. This study will also evaluate the community-level impact of DAA treatment scale-up on HCV incidence and prevalence.

11.7 Monitoring and Evaluation of HCV Elimination

Given rapid initial DAA uptake, Australia would appear to be "on-track" to achieve WHO HCV elimination targets by 2030. Ongoing monitoring and evaluation is, however, crucial to inform further HCV elimination strategic development. The key elements of this program will assess DAA uptake, treatment outcomes, and the population-level impact on HCV prevalence, incidence, and disease burden.

From the start of the Australian DAA program in March 2016, a monitoring of DAA treatment uptake has been undertaken, with regular newsletters to inform stakeholders of progress [9, 45]. These newsletters provided data of DAA treatment initiation numbers per month and, in each jurisdiction, demographic details of patients (age and gender distribution), DAA regimens, and prescriber pattern.

Two large-scale observational cohort studies are undertaken to evaluate real-world outcomes of DAA therapy in Australia. *Observational Prospective Epidemiological Registry in Australia of HCV (OPERA-C)* largely recruits from tertiary care gastroenterology and liver clinics, and *Real world Efficacy of Antiviral therapy in Chronic Hepatitis C (REACH-C)* collects data from tertiary care, primary care, drug and alcohol, and prison-based clinics. Initial DAA treatment outcomes among 4223 patients from REACH-C have been reported [46], with a per protocol SVR of 96%. Similar to many other real-world cohorts [47–50], there is a sizable rate of loss to follow-up between end of treatment and SVR at 12 weeks post-treatment (16%), highlighting the need to optimise ongoing patient engagement. The REACH-C cohort will also evaluate rates and outcomes of HCV retreatment, both for virologic failure and reinfection.

Data linkage studies are major components of disease burden monitoring, linking notified HCV cases with several administrative datasets, including individual-level Pharmaceutical Benefits Scheme DAA treatment, hospitalisation, cancer diagnoses, and death. These studies have characterised population-level burden of decompensated cirrhosis, HCC, and liver-related mortality [6, 7, 51–55] and will continue to monitor this burden and the specific impact of DAA treatment scale-up in Australia. These studies will inform the progress towards the WHO HCV elimination target of 65% reduction in liver-related mortality by 2030. They will also provide essential information to validate and/or adjust mathematical model-based estimates and projections of the impact of DAA therapy on advanced liver disease complications and mortality.

Ongoing surveillance through the Australian Needle and Syringe Program Survey will be a major component of HCV elimination monitoring. The inclusion from 2015 of HCV RNA testing on dried blood spot samples provides the ideal format for PWID-based HCV viraemic estimates over time. A comparison of 2015 and 2017 samples demonstrated a reduction in HCV RNA prevalence from 43% to 25% [22], strong evidence for an initial population-level impact of DAA treatment.

Fortunately, HCV has been a mandatory notifiable disease in Australia since the early 1990s, with established HCV notification registries at jurisdictional (state and territory) and national level. National HCV surveillance data will provide further valuable information. Monitoring of rates of new HCV diagnosis among younger

age groups (e.g. 15–24 years), which have been stable over recent years, should provide a surrogate measure of recent HCV transmission trends. High rates of HCV screening among high-risk populations, particularly PWID, provide reassurance that this form of surveillance is valuable in terms of monitoring HCV transmission. The linkage of HCV notifications to several administrative datasets, as described above, also provides the opportunity to evaluate timing between HCV diagnosis and presentation with advanced liver disease complications (decompensated cirrhosis and hepatocellular carcinoma). This has enabled monitoring of the proportion of individuals with "late HCV diagnosis", defined as HCV diagnosis less than 2 years prior to these complications. This proportion has progressively declined to around 20% by the early 2010s, suggesting that a large pool of undiagnosed people is not present in the population, at least among those with prolonged infection [52].

11.8 Moving Forward

There are a number of key challenges that will need to be met, if Australia is to continue on the path to HCV elimination. Continued efforts are required to sustain ongoing DAA uptake, even at lower levels than 2016–2017. There may be a tendency to consider the HCV public health "problem" solved, given the high DAA cure rates, major Australian Government investment in unrestricted DAA access, and the initial extremely encouraging uptake. Continued advocacy will therefore be required from a range of stakeholders to provide further investment in DAA treatment implementation. The prison setting provides an opportunity for further scale-up and access to highly marginalised individuals from the community. Innovative DAA treatment programs, including delivery through needle and syringe program services with peer-based support, need to be evaluated as a means to extending DAA therapy reach for current PWID. The broad prescriber base is encouraging, but an even larger pool of clinicians, particularly those with a potentially high caseload of people with HCV, needs to be developed. Community awareness needs to be further elevated, particularly to enable those individuals not regularly engaged in drug and alcohol services (e.g. most former PWID). Considerable communication is happening on the ground, through social and injecting networks, following tens of thousands of people being cured in the last 2 years, but more formal education and awareness raising is also required. Finally, the central role of harm reduction, including needle syringe programs and access to opioid substitution therapy, in HCV elimination should not be forgotten. Without a strong HCV "prevention as prevention" foundation, DAA therapy will not provide the population-level impact required to achieve major reductions in HCV incidence required to achieve WHO elimination targets.

Australia has a wonderful opportunity to provide international leadership on many aspects of HCV elimination. Broad access to DAA therapy provides considerable empowerment, for individuals living with HCV who have extremely high prospects for cure and for healthcare professionals to be involved in frequent cure

of a chronic disease. There is a clear need to harness this potential empowerment to continue the drive towards HCV elimination.

References

1. Razali K, Thein HH, Bell J, et al. Modelling the hepatitis C virus epidemic in Australia. Drug Alcohol Depend. 2007;91(2–3):228–35.
2. The Kirby Institute. National update on HIV, viral hepatitis and sexually transmissible infections in Australia: 2009–2018. Sydney, Australia: The Kirby Institute, UNSW Sydney; 2020.
3. Hajarizadeh B, Grebely J, McManus H, et al. Chronic hepatitis C burden and care cascade in Australia in the era of interferon-based treatment. J Gastroenterol Hepatol. 2017;32(1):229–36.
4. Sievert W, Razavi H, Estes C, et al. Enhanced antiviral treatment efficacy and uptake in preventing the rising burden of hepatitis C-related liver disease and costs in Australia. J Gastroenterol Hepatol. 2014;29:1–9.
5. Alavi M, Janjua NZ, Chong M, et al. Trends in hepatocellular carcinoma incidence and survival among people with hepatitis C: an international comparison. J Viral Hepat. 2018;25(5):473–81.
6. Waziry R, Grebely J, Amin J, et al. Trends in hepatocellular carcinoma among people with HBV or HCV notification in Australia (2000–2014). J Hepatol. 2016;65(6):1086–93.
7. Alavi M, Law MG, Grebely J, et al. Lower life expectancy among people with an HCV notification: a population-based linkage study. J Viral Hepat. 2014;21(6):e10–e8.
8. The Kirby Institute. HIV, viral hepatitis and sexually transmissible infections in Australia: Annual Surveillance Report 2016. Sydney NSW: The Kirby Institute, UNSW Australia; 2016.
9. Hajarizadeh B, Grebely J, Matthews GV, Martinello M, Dore GJ. Uptake of direct acting antiviral treatment for chronic hepatitis C in Australia. J Viral Hepat. 2018;25(6):640–8.
10. Australian Government Department of Health and Ageing. Fifth National Hepatitis C Strategy 2018–2022. Canberra: Commonwealth of Australia. 2018. https://www1.health.gov.au/internet/main/publishing.nsf/Content/ohp-bbvs-1
11. Hepatitis C Virus Infection Consensus Statement Working Group. Australian recommendations for the management of hepatitis C virus infection: a consensus statement (June 2020). Gastroenterological Society of Australia, Melbourne, Australia, 2020.
12. Baker D, Alavi M, Erratt A, et al. Delivery of treatment for hepatitis C virus infection in the primary care setting. Eur J Gastroenterol Hepatol. 2014;26(9):1003–9.
13. Butler K, Day C, Sutherland R, et al. Hepatitis C testing in general practice settings: a cross-sectional study of people who inject drugs in Australia. Int J Drug Policy. 2017;47(Supplement C):102–6.
14. Dore GJ, Hajarizadeh B. Elimination of HCV in Australia: laying the foundation. Infect Dis Clin N Am. 2018;32(2):269–79.
15. Larney S, Peacock A, Leung J, et al. Global, regional, and country-level coverage of interventions to prevent and manage HIV and hepatitis C among people who inject drugs: a systematic review. Lancet Glob Health. 2017;5(12):e1208–e20.
16. Iversen J, Wand H, Topp L, Kaldor J, Maher L. Extremely low and sustained HIV incidence among people who inject drugs in a setting of harm reduction. AIDS. 2014;28(2):275–8.
17. Iversen J, Grebely J, Catlett B, Cunningham P, Dore GJ, Maher L. Estimating the cascade of hepatitis C testing, care and treatment among people who inject drugs in Australia. Int J Drug Policy. 2017;47:77–85.
18. Barua S, Greenwald R, Grebely J, Dore GJ, Swan T, Taylor LE. Restrictions for medicaid reimbursement of sofosbuvir for the treatment of hepatitis C virus infection in the United States medicaid restrictions of sofosbuvir for hepatitis C. Ann Intern Med. 2015;163(3):215–23.

19. Marshall AD, Cunningham EB, Nielsen S, et al. Restrictions for reimbursement of interferon-free direct-acting antiviral drugs for HCV infection in Europe. Lancet Gastroenterol Hepatol. 2018;3(2):125–33.
20. World Health Organization. Global heath strategy on viral hepatitis 2016-2021 towards ending viral hepatitis. Geneva, Switzerland: World Health Organization; 2016.
21. Kwon A, Dore G, Grebely J, et al. Australia on track to achieve WHO HCV elimination targets following rapid initial DAA treatment uptake: a modeling study. J Viral Hepat. 2019;26(1):83–92.
22. Iversen J, Dore G, Catlett B, Cunningham P, Grebely J, Maher L. Association between rapid utilisation of direct hepatitis C antivirals and decline in the prevalence of viremia among people who inject drugs in Australia. J Hepatol. 2018;70(1):33–9.
23. Heard S, Iversen J, Geddes L, Maher L. Australian NSP survey: Prevalence of HIV, HCV and injecting and sexual behaviour among NSP attendees, 25-year National Data Report 1995–2019. Sydney, Australia: The Kirby Institute, UNSW Sydney; 2020.
24. Iranpour N, Dore GJ, Martinello M, Matthews GV, Grebely J, Hajarizadeh B. Estimated uptake of hepatitis C direct-acting antiviral treatment among individuals with HIV coinfection in Australia: a retrospective cohort study. Sexual Health. 2020;17(3):223–30.
25. Rodgers C. The kirketon road centre improving access to primary care for marginalised populations. Aust Fam Physician. 2012;41:245–7.
26. van Beek I. Case study: accessible primary health care—a foundation to improve health outcomes for people who inject drugs. Int J Drug Policy. 2007;18(4):329–32.
27. Read P, Lothian R, Chronister K, et al. Delivering direct acting antiviral therapy for hepatitis C to highly marginalised and current drug injecting populations in a targeted primary health care setting. Int J Drug Policy. 2017;47:209–15.
28. Larney S, Kopinski H, Beckwith CG, et al. Incidence and prevalence of hepatitis C in prisons and other closed settings: Results of a systematic review and meta-analysis. Hepatology. 2013;58(4):1215–24.
29. Butler T, Simpson M. National prison entrants' blood-borne virus survey report 2004, 2007, 2010, 2013, and 2016. Sydney, Australia: Kirby Institute, UNSW Sydney; 2017.
30. Lloyd AR, Clegg J, Lange J, et al. Safety and effectiveness of a nurse-led outreach program for assessment and treatment of chronic hepatitis C in the custodial setting. Clin Infect Dis. 2013;56(8):1078–84.
31. Wade AJ, Macdonald DM, Doyle JS, et al. The cascade of care for an Australian community-based hepatitis C treatment service. PLoS One. 2015;10(11):e0142770.
32. Cheng W, Nazareth S, Flexman JP. Statewide hepatitis C model of care for rural and remote regions. J Gastroenterol Hepatol. 2015;30:1–5.
33. Doyle JS, van Santen DK, Iser D, et al. Microelimination of hepatitis C among people with human immunodeficiency virus coinfection: declining incidence and prevalence accompanying a multicenter treatment scale-up trial. Clin Infect Dis. 2020. ciaa1500, https://doi.org/10.1093/cid/ciaa1500
34. Grebely J, Alavi M, Micallef M, et al. Treatment for hepatitis C virus infection among people who inject drugs attending opioid substitution treatment and community health clinics: the ETHOS Study. Addiction. 2015;111(2):311–9.
35. Treloar C, Newland J, Rance J, Hopwood M. Uptake and delivery of hepatitis C treatment in opiate substitution treatment: perceptions of clients and health professionals. J Viral Hepat. 2010;17(12):839–44.
36. Valerio H, Alavi M, Silk D, et al. Progress towards elimination of hepatitis C infection among people who inject drugs in Australia: the ETHOS Engage Study. Clin Infect Dis. 2020. ciaa571, https://doi.org/10.1093/cid/ciaa571
37. Razavi H, Sanchez Gonzalez Y, Yuen C, Cornberg M. Global timing of hepatitis C virus elimination in high-income countries. Liver International. 2020;40(3):522–9.

38. Scott N, McBryde ES, Thompson A, Doyle JS, Hellard ME. Treatment scale-up to achieve global HCV incidence and mortality elimination targets: a cost-effectiveness model. Gut. 2017;66(8):1507–15.
39. Larney S, Hickman M, Guy R, et al. Estimating the number of people who inject drugs in Australia. BMC Public Health. 2017;17:757.
40. Scott N, Doyle JS, Wilson DP, et al. Reaching hepatitis C virus elimination targets requires health system interventions to enhance the care cascade. Int J Drug Policy. 2017;47:107–16.
41. Hajarizadeh B, Grebely J, Byrne M, et al. Reduced hepatitis C incidence associated with rapid treatment scale-up in Australian prisons: treatment-as-prevention in the SToP-C study. Available at SRN: https://ssrn.com/abstract=3709824 or https://doi.org/10.2139/ssrn.3709824
42. Hellard M, McBryde E, Sacks Davis R, et al. Hepatitis C transmission and treatment as prevention—the role of the injecting network. Int J Drug Policy. 2015;26(10):958–62.
43. Martinello M, Yee J, Bartlett SR, et al. Moving towards hepatitis C microelimination among people living with human immunodeficiency virus in Australia: the CEASE study. Clin Infect Dis. 2019;71(6):1502–10.
44. The Kirby Institute. Blood-borne viral and sexually transmissible infections in Aboriginal and Torres Strait Islander people: annual surveillance report 2016. Sydney, NSW: The Kirby Institute, UNSW Sydney; 2016.
45. The Kirby Institute. Monitoring hepatitis C treatment uptake in Australia (Issue 10). Sydney, Australian: The Kirby Institute, UNSW Sydney; 2019. https://kirby.unsw.edu.au/report/monitoring-hepatitis-c-treatment-uptake-australia-issue-10-june-2019.
46. The Kirby Institute, UNSW Sydney. Real world efficacy of antiviral therapy in chronic hepatitis C in Australia (Issue 2). July 2018. https://kirby.unsw.edu.au/report/real-world-efficacy-antiviral-therapy-chronic-hepatitis-c-reach-c-australia-issue-2-july-2018.
47. Backus LI, Belperio PS, Shahoumian TA, Loomis TP, Mole LA. Real world effectiveness of ledipasvir/sofosbuvir in 4365 treatment-Naïve genotype 1 hepatitis C infected patients. Hepatology. 2016;64(2):405–14.
48. Calleja JL, Crespo J, Rincón D, et al. Effectiveness, safety and clinical outcomes of direct-acting antiviral therapy in HCV genotype 1 infection: results from a Spanish real world cohort. J Hepatol. 2017;66(6):1138–48.
49. Ioannou GN, Beste LA, Chang MF, et al. Effectiveness of sofosbuvir, ledipasvir/sofosbuvir, or paritaprevir/ritonavir/ombitasvir and dasabuvir regimens for treatment of patients with hepatitis C in the veterans affairs national health care system. Gastroenterology. 2016;151(3):457–71. e5.
50. Terrault NA, Zeuzem S, Di Bisceglie AM, et al. Effectiveness of ledipasvir-sofosbuvir combination in patients with hepatitis C virus infection and factors associated of sustained virologic response. Gastroenterology. 2016;151(6):1131–40. e5.
51. Alavi M, Janjua NZ, Chong M, et al. The contribution of alcohol use disorder to decompensated cirrhosis among people with hepatitis C: an international study. J Hepatol. 2018;68(3):393–401.
52. Alavi M, Law MG, Grebely J, et al. Time to decompensated cirrhosis and hepatocellular carcinoma after an HBV or HCV notification: a population-based study. J Hepatol. 2016;65:879–87.
53. Aspinall EJ, Hutchinson SJ, Janjua NZ, et al. Trends in mortality after diagnosis of hepatitis C virus infection: An international comparison and implications for monitoring the population impact of treatment. J Hepatol. 2015;62(2):269–77.
54. Waziry R, Grebely J, Amin J, et al. Survival following hospitalization with hepatocellular carcinoma among people notified with hepatitis B or C virus in Australia (2000–2014). Hepatol Commun. 2017;1(8):736–47.
55. Alavi M, Law MG, Valerio H, et al. Declining hepatitis C virus-related liver disease burden in the direct-acting antiviral therapy era in New South Wales, Australia. J Hepatol. 2019;71(2):281–8.

Egypt: Towards Successful Elimination of HCV in Low-Income Countries

12

Heba Omar, Tamer Elbaz, and Gamal Esmat

Hepatitis C virus (HCV) infection is a major public challenge that affects more than 71 million people all over the world [1]. Egypt is one of the world's highest prevalence rates of HCV infection. According to Egyptian Demographic Health Survey (EDHS) conducted in 2008, a substantial proportion of Egyptian population was affected by the virus, with an overall estimate of HCV antibody and HCV RNA positivity among the 15–59-year age group of 14.7% and 9.9%, respectively [2]. In 2015, the Egyptian Health Issues Survey (EHIS) was done to re-estimate the prevalence of HCV infection in Egypt, HCV population burden was estimated by around six million patients, and the overall prevalence of HCV antibody and HCV RNA positivity among 15–59-years-age group went down to 10.0% and 7.0%, respectively [3], with 29% reduction in HCV RNA seroprevalence since 2008. The decline in HCV prevalence, although looks encouraging, is not pure decline; stock of patients who were infected due to bilharzial treatment are age shifted so that majority of them are now older than 60 years so are not included in 2015 survey while were included in 2008 survey. Also, no obvious impact of direct-acting antivirals (DAAs) was expected although it is believed that a related true decline will be proved in future surveys.

The burden of HCV infection in Egypt is largely attributed to the community-wide campaigns that were conducted by the Egyptian Ministry of Health (EOH) during the period from 1950 to 1980, aiming at the eradication of schistosomiasis which was the major public health problem and a major cause of liver disease at that time [4–6]. This great effort gave rise to a huge reservoir of HCV genotype four in the country due to exposure of the patients to unsafe injection practices [6]. In addition, health-care related transmission affects about 150,000 Egyptian patients per year [7].

H. Omar · T. Elbaz · G. Esmat (✉)
Faculty of Medicine, Endemic Medicine and Hepato-gastroenterology Department, Cairo University, Cairo, Egypt

A. Hatzakis (ed.), *Hepatitis C: Epidemiology, Prevention and Elimination*,
https://doi.org/10.1007/978-3-030-64649-3_12

HCV may complicate the course of schistosomiasis and vice versa (possibly through synergistic effect). Liver cirrhosis occurs at a much higher rate in those who have co-infection of HCV with schistosomiasis (48%), compared with those who are mono-infected with either HCV or schistosomiasis (15% and 0%, respectively) [8].

HCV is a small (55–65 nm in size), enveloped, strictly blood-borne RNA virus in Flaviviridae family [9]. It can survive infectious in dried blood for weeks [10]. The major routes for its spread include injection drug use with a shared unsterilized needle, medical and dental procedures in settings with inadequate infection control, body piercing with reused needles, and transfusion of unscreened blood or blood products. Less common modes of HCV spread include sexual transmission and viral transmission from an infected mother to her baby [11]. In Egypt, approximately 150,000 individuals acquire HCV infection annually primarily through a healthcare-related transmission which is considered the primary mode of HCV transmission in Egypt [7].

Nearly 75%–85% of HCV-infected patients do not clear infection within 6 months, and subsequently, they develop chronic hepatitis [8, 12]. Many factors affect the rate of HCV chronicity, including patients' age at the time of infection, gender, ethnicity, and the development of jaundice during the acute phase of infection [13].

Vulnerable groups for acquiring HCV infection, to which screening programs are directed, include healthcare, emergency medical, or public safety workers who are in contact with HCV-positive blood, injection drug users, patients who received a blood transfusion or organ transplant before July 1992 or clotting factor concentrates produced before 1987, long-term hemodialysis patients, and babies born to HCV-positive mothers.

The journey of Egyptian battle against hepatitis C started in 2001 by the great efforts of Egyptian Ministry of Health (MOH) in the execution of a multitask strategic program aiming at reduction of HCV transmission in Egypt. The program involved the foundation of the first HCV surveillance unit at the MOH as well as the release of the first national guidelines for HCV infection control. Both actions contributed to reduction in the annual incidence of de novo HCV infection among dialysis patients from 28% to 6% in 2008 [14].

The second major achievement for the Egyptian Ministry of Health (MOH) in regard to HCV infection control was the establishment of the National Committee for Control of Viral Hepatitis (NCCVH) in 2006 in collaboration with ad hoc experts from the University of California in San Francisco (United States) and Pasteur Institute in Paris (France) [15, 16]. The NCCVH set its mission to eradicate HCV in Egypt by 2030 through setting up, implementing, and maintaining a nationwide HCV control strategy aiming at the reduction of the infection rate from currently 8% to 2% (international prevalence disease). NCCVH also offers antiviral medications for HCV-infected patients at minimum cost or even totally free of charge. Nowadays, patients with viral hepatitis are served through more than 60 treatment centers all over the country [17].

12.1 Strategic and Action Plan Evolution During Egyptian Journey Towards HCV Elimination

"Elimination of HCV in Egypt is critically dependent on the prevention of new infections, through the adoption of universal infection control strategies at all levels of the health system hand in hand with the implementation of universal screening program and treatment of all diagnosed cases."

12.1.1 Addressing HCV Problem and Raising the Awareness Against HCV Transmission

In 2011, the national services for awareness against HCV were limited to few media-based public awareness campaigns and educational campaigns targeting university students (nearly 100,000 university undergraduates in different areas). During the period between 2014 and 2018, the NCCVH adopted a novel "Action Plan" addressing all aspects of control of viral hepatitis with support from the World Health Organization (WHO), the US Centers for Disease Control and Prevention (US-CDC), and the Pasteur Institute, Paris. The "Action Plan" strategies were to improve current HCV surveillance practices, ensure blood and blood products safety, promote infection control measures, increase public awareness about viral hepatitis prevention, and improve care and treatment of viral hepatitis-related liver disease and cancer [16].

12.1.2 Implementing Universal Screening

Eradication of HCV is a possible target since the disease meets most of the criteria for disease eradicability [18, 19]. However, such target would not be achievable without improvement of screening effectiveness [20]. The current risk-based screening approach is problematic since a significant number of patients are unaware of their infection status [21]. This underdiagnosis comprises a missed opportunity for infected patients to benefit from early access to treatment and allows the spread of infection from the untreated patient (untreated patient could transmit the infection to an average of three to four patients during his lifetime). Therefore, an extension of risk-based screening strategy to universal screening could possibly increase the detection rate of undiagnosed HCV cases and subsequently significantly reduces the disease burden.

Great efforts have been made in that context; Cairo University launched regular screening and awareness campaigns for its workers and students. The innovative initiatives from professor Gamal Shiha (towards a free village from viral hepatitis 2014–2019) establishing exemplary village free from viral hepatitis through implementing community-based outreach interventions and provision of accessible and comprehensive treatment and care services that address HCV and the steps taken by the NCCVH to unmask the hidden part of unrevealed HCV patients by screening

different populations and relatives of diagnosed HCV patients are good examples of such efforts.

Media awareness campaigns were launched on radio and TV to increase awareness about this hidden epidemic and encourage people to get tested for hepatitis C in collaboration with community leaders and social stars.

12.1.3 Treating HCV Patients and Ending Hepatitis C Transmission with Antiviral Medication

Biologically, HCV is an amenable target for eradication as there is no known non-human reservoir as well as no latent cellular reservoir [22]; thus, treatment-based elimination of HCV could be an option [23]. The most prevalent HCV RNA genotype in Egypt is genotype 4, accounting for >90% of all HCV cases [24].

NCCVH issues the national treatment protocols and generalizes it to all its affiliated specialized treatment centers. These treatment protocols are regularly updated by expert hepatologist panel regarding patients' treatment eligibility criteria, pretreatment assessments, drug regimens, and treatment of special populations.

12.2 Revulsions of Patients' Assessments in NCCVH Affiliated Centers

- Initial pretreatment evaluation of hepatic fibrosis stage based on FibroScan; now NCCVH affiliated centers depend on FIB4 score to assess patients' fibrosis stage.
- The target population for HCV treatment was initially patient with fibrosis stages 3 and 4 being the most vulnerable group for HCV-related complications and hepatic decompensation. Currently, treatment of HCV is offered to all HCV-infected patients regardless the fibrosis stage aiming at the reduction of infection transmission.
- Before the era of direct acting antivirals (DAA), between 2007 and 2014, the available choices for treatment of HCV were very limited. The NCCVH offered HCV- infected patients with a combination of pegylated interferon (Peg IFN) and ribavirin (RBV) which was the standard of care (SOC) at that time, leading to a sustained virologic response (SVR) that didn't reach 65%. The treatment options for HCV have been revolutionized since the introduction of DAAs on 2014 to a highly efficacious and well-tolerated therapy for nearly all HCV-infected patients with a significant increment in SVR rates from 40 to 50% with Peg IFN/RBV [25] to higher than 90% response rates. In real-life experience of Egyptian NCCVH with DAAs, a mass treatment plan [14] that basically depended on sofosbuvir-based regimen using its combination with ribavirin (dual therapy) or Peg IFN/RBV (triple therapy) was implemented in September 2014 with SVR-12 rates for those who received dual ($n = 5667$) and triple therapy ($n = 8742$) as 79% and 94%, respectively. Between May 2015 and November 2015, 6211 patients received combined sofosbuvir and simeprevir therapy and achieved 94% SVR-

12 [26]. Since November 2015, the protocol of NCCVH is directed to treat HCV patients with two DAAs ± RBV. Since that time, sofosbuvir/daclatasvir regimen with or without ribavirin took the lead of management ($n = 18{,}378$) with an overall SVR-12 of 95.08%. Sofosbuvir/ledipasvir with or without ribavirin and paritaprevir/ritonavir/ombitasvir + ribavirin regimens are also available.

– The concern about HCC recurrence in patients treated with DAAs for hepatitis C following curative tumor therapy reflected on NCCVH treatment protocol for this subset of patients. Patients with HCV-related hepatocellular carcinoma are currently eligible to receive treatment for their HCV with DAA after completing 6 months after successful curative interventions that aimed complete HCC ablation and so concurrent no evidence of activity by dynamic imaging (CT, MRI) before starting HCV treatment.

12.3 Addressing DAA Availability

DAAs have considered the ideal choice for treatment of chronic HCV infection being effective medications (SVR > 90%) with minimal adverse effects. The main barrier to universal use of DAA treatment has stemmed from their exorbitant prices; in the United States, a 12-week course of treatment with sofosbuvir and simeprevir costs around USD84,000 and USD66,000, respectively [27]. Egypt took many steps to obtain the widest possible treatment coverage for Egyptian HCV-infected patients. The negotiation between the government in the form of "NCCVH" and Gilead sciences resulted in the importation of Gilead's sofosbuvir (Sovaldi) in 1% of its original price and sofosbuvir/ledipasvir (Harvoni). Gilead also allowed 11 Indian and 2 Egyptian pharmaceutical companies to make sofosbuvir under license and to price it as they like. In real-life experience, many Egyptian studies concluded that the efficacy of generic drugs in Egyptian population was proven to be comparable to brand one [28].

The NCCVH tests the effectiveness of each individual new DAA before its rollout in the Egyptian market through conduction of multicenter, randomized, active-controlled studies in its well-equipped hepatology centers. The results of those trials are analyzed and published in order to give a pure view on the effectiveness of the candidate medication in treatment of HCV genotype 4 in Egypt.

12.4 It Could Be Very Soon

In 2014, the government set ambitious goals to increase the number of newly diagnosed cases from 150,000/year in 2015 to 340,000/year in 2018 and to target treatment of 325,000 patients annually from 2018. By that, Egypt might be able to achieve hepatitis C elimination by 2030 [29]. Eventually, with a marked actual jump in the numbers of really treated patients, eradication of HCV could be achieved earlier in time.

References

1. World Health Organization. Hepatitis C [online]. 2018. http://www.who.int/en/news-room/fact-sheets/detail/hepatitis-c. Accessed 1 Jun 2018.
2. El-Zanaty F, Way A. Egypt demographic and health survey 2008. Cairo, Egypt: Ministry of Health and Population; 2009.
3. Ministry of Health, Egypt, El-Zanaty and Associates, Egypt and ICF International. Egypt Health Issues Survey 2015. Cairo, Egypt/Rockville, MD: Ministry of Health and ICF International; 2015.
4. Strickland G. Liver disease in Egypt: hepatitis C superseded schistosomiasis as a result of iatrogenic and biological factors. Hepatology. 2006;43(5):915–22.
5. Rao M, Naficy A, Darwish M, Darwish N, Schisterman E, Clemens J, Edelman R. Further evidence for association of hepatitis C infection with parenteral schistosomiasis treatment in Egypt. BMC Infect Dis. 2002;2(1):29.
6. Frank C, Mohamed M, Strickland G, Lavanchy D, Arthur R, Magder L, Khoby T, Abdel-Wahab Y, Ohn E, Anwar W, Sallam I. The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. Lancet. 2000;355(9207):887–91.
7. Hajarizadeh B, Grebely J, Dore G. Epidemiology and natural history of HCV infection. Nat Rev Gastroenterol Hepatol. 2013;10(9):553–62.
8. Struthers A. From schistosomiasis to hepatitis C: the spread of HCV in Egypt. Med J Therapeut Africa. 2007;3:213–21. http://mjota.org/images/SpreadofHCVEgypt.pdf. Accessed 16 Feb 2016.
9. Miller F, Abu-Raddad L. Evidence of intense ongoing endemic transmission of hepatitis C virus in Egypt. Proc Natl Acad Sci. 2010;107(33):14757–62.
10. Paintsil E, Binka M, Patel A, Lindenbach B, Heimer R. Hepatitis C virus maintains infectivity for weeks after drying on inanimate surfaces at room temperature: implications for risks of transmission. J Infect Dis. 2013;209(8):1205–11.
11. Yeung C. Vertical transmission of hepatitis C virus: current knowledge and perspectives. World J Hepatol. 2014;6(9):643.
12. Hoofnagle J. Hepatitis C: the clinical spectrum of disease. Hepatology. 1997;26(S3):15S–20S.
13. Chen S, Morgan T. The natural history of hepatitis C virus (HCV) infection. Int J Med Sci. 2006;3:47–52.
14. CDC C. Progress toward prevention and control of hepatitis C virus infection—Egypt, 2001–2012.—PubMed-NCBI. [online] 2018. Ncbi.nlm.nih.gov. https://www.ncbi.nlm.nih.gov/pubmed/22832935. Accessed 1 Jun 2018.
15. El-Akel W, El-Sayed M, El Kassas M, El-Serafy M, Khairy M, Elsaeed K, Kabil K, Hassany M, Shawky A, Yosry A, Shaker M, ElShazly Y, Waked I, Esmat G, Doss W. National treatment programme of hepatitis C in Egypt: hepatitis C virus model of care. J Viral Hepat. 2017;24(4):262–7.
16. Gomaa A, Allam N, Elsharkawy A, El Kassas M, Waked I. Hepatitis C infection in Egypt: prevalence, impact and management strategies [Corrigendum]. Hepat Med Evid Res. 2017;9:35–6.
17. El Kassas M, Elbaz T, Elsharkawy A, Omar H, Esmat G. HCV in Egypt, prevention, treatment and key barriers to elimination. Expert Rev Anti-Infect Ther. 2018;16(4):345–50.
18. Hellard M, Doyle J, Sacks-Davis R, Thompson A, McBryde E. Eradication of hepatitis C infection: the importance of targeting people who inject drugs. Hepatology. 2013;59(2):366–9.
19. Hopkins D. Disease Eradication. N Engl J Med. 2013;368(1):54–63.
20. Wedemeyer H, Duberg A, Buti M, Rosenberg W, Frankova S, Esmat G, Örmeci N, Van Vlierberghe H, Gschwantler M, Akarca U, Aleman S, Balık İ, Berg T, Bihl F, Bilodeau M, Blasco A, Brandão Mello C, Bruggmann P, Calinas F, Calleja J, Cheinquer H, Christensen P, Clausen M, Coelho H, Cornberg M, Cramp M, Dore G, Doss W, El-Sayed M, Ergör G, Estes C, Falconer K, Félix J, Ferraz M, Ferreira P, García-Samaniego J, Gerstoft J, Giria J, Gonçales F, Guimarães Pessôa M, Hézode C, Hindman S, Hofer H, Husa P, Idilman R, Kåberg M, Kaita K,

Kautz A, Kaymakoglu S, Krajden M, Krarup H, Laleman W, Lavanchy D, Lázaro P, Marinho R, Marotta P, Mauss S, Mendes Correa M, Moreno C, Müllhaupt B, Myers R, Nemecek V, Øvrehus A, Parkes J, Peltekian K, Ramji A, Razavi H, Reis N, Roberts S, Roudot-Thoraval F, Ryder S, Sarmento-Castro R, Sarrazin C, Semela D, Sherman M, Shiha G, Sperl J, Stärkel P, Stauber R, Thompson A, Urbanek P, Van Damme P, van Thiel I, Vandijck D, Vogel W, Waked I, Weis N, Wiegand J, Yosry A, Zekry A, Negro F, Sievert W, Gower E. Strategies to manage hepatitis C virus (HCV) disease burden. J Viral Hepat. 2014;21:60–89.

21. Denniston M, Klevens R, McQuillan G, Jiles R. Awareness of infection, knowledge of hepatitis C, and medical follow-up among individuals testing positive for hepatitis C: National Health and Nutrition Examination Survey 2001–2008. Hepatology. 2012;55(6):1652–61.
22. Shop.lww.com. Fields Virology. [online]. 2018. https://shop.lww.com/Fields-Virology/p/9781451105636. Accessed 1 Jun 2018.
23. Pawlotsky J. New hepatitis C therapies: the toolbox, strategies, and challenges. Gastroenterology. 2014;146(5):1176–92.
24. Ray S, Arthur R, Carella A, Bukh J, Thomas D. Genetic epidemiology of hepatitis C virus throughout Egypt. J Infect Dis. 2000;182(3):698–707.
25. Asselah T, De Muynck S, Broët P, Masliah-Planchon J, Blanluet M, Bièche I, Lapalus M, Martinot-Peignoux M, Lada O, Estrabaud E, Zhang Q, El Ray A, Vidaud D, Ripault M, Boyer N, Bedossa P, Valla D, Vidaud M, Marcellin P. IL28B polymorphism is associated with treatment response in patients with genotype 4 chronic hepatitis C. J Hepatol. 2012;56(3):527–32.
26. Eletreby R, Elakel W, Said M, El Kassas M, Seif S, Elbaz T, El Raziky M, Abdel Rehim S, Zaky S, Fouad R, Gamal Eldeen H, Abdo M, Korany M, Yosry A, El Serafy M, El-Sayed M, ElShazly Y, Waked I, Doss W, Esmat G. Real life Egyptian experience of efficacy and safety of Simeprevir/Sofosbuvir therapy in 6211 chronic HCV genotype IV infected patients. Liver Int. 2016;37(4):534–41.
27. Project Inform. Fair pricing coalition condemns Gilead for exorbitant price of new hep C drug—Project Inform. [online]. 2018. https://www.projectinform.org/hepc/fair-pricing-coalition-condemns-gilead-for-exorbitant-price-of-new-hep-c-drug/. Accessed 1 Jun 2018.
28. P150: Effect of Sofosbuvir. Brand drug (Sofaldi) versus generic (Viropack) in treating chronic HCV infection among Egyptian patients. J Viral Hepat. 2015;22:94.
29. Waked I, Doss W, El-Sayed M, Estes C, Razavi H, Shiha G, Yosry A, Esmat G. The current and future disease burden of chronic hepatitis C virus infection in Egypt. Arab J Gastroenterol. 2014;15(2):45–52.

National Hepatitis C Elimination Program of Georgia

13

Tengiz Tsertsvadze, David Sergeenko, Amiran Gamkrelidze, Nikoloz Chkhartishvili, Maia Butsashvili, David Metreveli, Akaki Abutidze, Lia Gvinjilia, Muazzam Nasrullah, and Francisco Averhoff

13.1 HCV Epidemiology in Georgia

Georgia is a small Eastern European country (population: 3.7 million people) situated in the Caucasus between Russia and Turkey. The country has the fifth highest prevalence of hepatitis C in the world with an estimated 5.4% of adult population (150,000 persons) living with chronic HCV infection [1, 2]. Studies in various populations show that people who inject drugs have highest anti-HCV

T. Tsertsvadze (✉)
Infectious Diseases, AIDS and Clinical Immunology Research Center, Tbilisi, Georgia

Ivane Javakhishvili Tbilisi State University, Tbilisi, Georgia
e-mail: tt@aidscenter.ge

D. Sergeenko
Ministry of Labour, Health and Social Affairs of Georgia, Tbilisi, Georgia

A. Gamkrelidze
National Center for Disease Control and Public Health, Tbilisi, Georgia
e-mail: a.gamkrelidze@ncdc.ge

N. Chkhartishvili · A. Abutidze
Infectious Diseases, AIDS and Clinical Immunology Research Center, Tbilisi, Georgia
e-mail: nc@aidscenter.ge

M. Butsashvili
Clinic Neolab, Tbilisi, Georgia

D. Metreveli
Mrcheveli Medical Center, Tbilisi, Georgia

L. Gvinjilia
CDC Foundation, Tbilisi, Georgia

M. Nasrullah · F. Averhoff
Centers for Diseases Control and Prevention, Atlanta, USA
e-mail: hij9@cdc.gov; fma0@cdc.gov

A. Hatzakis (ed.), *Hepatitis C: Epidemiology, Prevention and Elimination*,
https://doi.org/10.1007/978-3-030-64649-3_13

Table 13.1 Hepatitis C burden in various populations in Georgia

Population	Anti-HCV+ (%)	HCV-RNA+ (%)
General population of Georgia [1]	7.7	5.4
General population of capital city Tbilisi [3]	6.7	N/A
People who inject drugs [4]	68.8	N/A
People who inject drugs [5]	63.2	N/A
Men who have sex with men [6]	7.2	N/A
People living with HIV [7]	40.3	34.3
People living with tuberculosis [8]	20.9	N/A
Healthcare workers [9]	5.0	N/A

N/A not available

prevalence of up to 70%, followed by people living with HIV (40%), people living with tuberculosis (21%), and others (Table 13.1).

According to the latest estimates, genotype 1 accounts for 41% of HCV infections in Georgia, followed by genotype 3, 35%, and genotype 2, 24%. There have been temporal changes in genotype distribution over the last 15-year period with increase in genotype 3 infections, primarily attributable to injection drug use [10, 11]. Interestingly, sequencing studies indicate that majority (about 70%) of genotype 2 infections in Georgia are actually recombinant form (RF) 2k/1b and thus may account for up to 18% of all infections in the country [12, 13]. This chimera virus possesses genotype 2 sequence in the structural and genotype 1 sequence in the non-structural region of the virus affecting response to antiviral therapy [14].

13.2 National Elimination Program

Georgia had been laying groundwork toward elimination for a long time through developing strong human and technical capacities and through increasing access to HCV therapy. Over the years, the Government of Georgia substantially stepped up its efforts against hepatitis C by implementing national programs such as free of charge hepatitis C treatment for HIV/HCV co-infection patients (implemented in collaboration with the Global Fund to Fight AIDS, TB, and Malaria since 2011) and free of charge hepatitis C treatment in the penitentiary system (2013) and negotiating 60% price reduction on combination of pegylated interferon and ribavirin for general population (2013).

These efforts culminated with the launch of world's first hepatitis C elimination program in April 2015 in partnership with US Centers for Disease Control and Prevention (CDC) and commitment from Gilead Sciences to donate its direct-acting antivirals (DAAs) to treat all Georgian living with HCV infection free of charge [15, 16]. Georgia has been chosen as a first model country for eliminating hepatitis C for several reasons, including:

- High prevalence of hepatitis and a small size of the country
- Strong political will and public support
- Strong technical and human capacities
- Existence of effective systems for implementing large-scale health programs
- Best practice experience in ensuring universal access to HIV and TB treatments

Combination of these factors strengthened by international partnership translated into successful rollout of elimination program. Together with CDC, WHO, and other international partners, Technical Advisory Group (TAG), represented by world's leading experts, was established to guide implementation of the program. Based on TAG recommendations, Georgia developed comprehensive strategic plan covering all key direction needed for eliminating hepatitis C by 2020, including advocacy and awareness; surveillance; prevention of transmission through blood safety, infection control, and harm reduction; and screening, care, and treatment. All these activities are implemented through either donor support or national allocations representing an example of an effective public–private partnership.

While Georgia's approach builds on delivering comprehensive response to HCV, treatment remains the cornerstone of elimination program. The overall goal of the program is to eliminate hepatitis C primarily through identifying and treating all HCV-positive persons strengthened by effective prevention interventions.

Despite very high effectiveness of modern DAAs approaching 100% cure rates, complete eradication of HCV infection, similar to that of smallpox, is impossible, and therefore Georgia set the goal for eliminating and not eradicating HCV. Although classical definition focuses on incidence [17], Georgia's HCV elimination goal was defined as 90% reduction in HCV prevalence from 5.4% to 0.5% [18].

To achieve the goal, the strategy has set forth 90-95-95 targets to be reached by 2020: (a) 90% of people living with HCV infection know their status; (b) 95% of people aware of their status are treated for HCV infection; and (c) 95% of people treated for HCV infection are cured.

Georgia's elimination program envisages active case finding and treating all patients, regardless of degree of liver damage, in order to achieve maximum prevention effect. Also for achieving the elimination goal, all patients with virological failure are retreated.

Treatment component of the elimination program started in April 2015 with four specialty clinics delivering care in the capital city of Tbilisi, and after 3 years, this expanded to over 30 HCV care provider clinics countrywide. Decentralization process further continues through establishing HCV treatment capacities in primary healthcare clinics and harm reduction sites.

Successful treatment expansion was possible through dedicated human capacity strengthening program delivered by Liver Institute and Foundation for Education and Research (L.I.F.E.R.) and Project ECHO of the New Mexico University.

National treatment protocols are developed in collaboration with leading international hepatologists and support simplified diagnostic and monitoring approaches. During the first year of the program, sofosbuvir (SOF) was the only DAA available within the program, which was used in combination with ribavirin with or without pegylated interferon. Since March 2016, Gilead donates fixed-dose combination of

ledipasvir/sofosbuvir (LDV/SOF). Exclusive decision was made for the elimination program to recommend LDV/SOF for all genotypes including with or without ribavirin for genotype 1 and in combination with ribavirin for genotypes 2 and 3.

Development of electronic health information systems has been essential part of elimination program. In 2015 national HCV treatment database was established, which is now modern web-based health information system connecting all HCV care providers countrywide. The database collects comprehensive case-based information, including demographic, laboratory, and clinical data, on every person enrolled in elimination program using standardized protocol. Effective validation mechanisms are available to ensure that high-quality data are captured. The database is the key source for monitoring treatment on individual and programmatic level, as well as for conducting research and for informing policies. In 2017 HCV screening database was launched to collect data from all sites providing HCV screening services in Georgia. The next step is to create unified system for hepatitis C elimination program integrated into the national e-health management system.

13.3 HCV Cascade and Treatment Outcomes

Figure 13.1 describes HCV care cascade as of March 31, 2018. After 3 years of program implementation, 32.5% of estimated number of people with chronic HCV infection were diagnosed; 93% of those diagnosed started treatment, and more than 98% of those assessed for sustained virologic response (SVR) cleared the virus, thus already exceeding treatment related 95% targets.

This cascade shows that success of the elimination program primarily depends on ability of the program to identify 90% of people living with HCV infection. Georgia responded to this challenge by scaling up screening, including through healthcare-based and outreach activities. As of March 31, 2018, over 974 thousand persons were screened for HCV (35% of adult population of Georgia), and one-third of the HCV-infected population were diagnosed. Analysis of the data showed the yield of

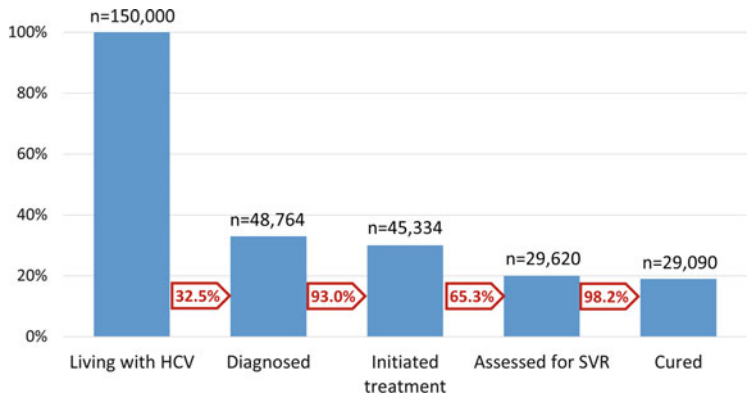


Fig. 13.1 HCV cascade as of March 31, 2018

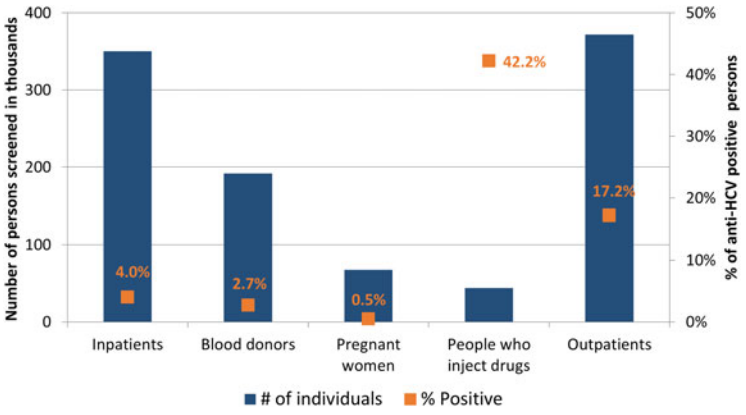


Fig. 13.2 HCV screening among different target groups

screening efforts differs between various populations: the highest rate of anti-HCV positivity of 42% was observed in harm reduction services for people who injected rugs, while only 0.5% tested positive in antenatal clinics (Fig. 13.2) [19]. This underlines the need for targeting services for those at highest risk of HCV infection. Together with international partners, the Ministry of Health of Georgia takes efforts to introduce innovative and high-quality strategies to increase awareness and improve access to screening.

During the initial year of the program, treatment was prioritized for patients with advanced liver damage (≥F3 METAVIR fibrosis score or FIB-4 score >3.25). Treatment initiation criteria expanded in June 2016 to treat all patients regardless of liver damage status. This resulted in 300% increase in treatment initiation rates peaking with 4552 persons starting treatment only in in August 2016. The rates declined afterward and flattened at monthly rate of around 1100 persons starting treatment in 2017 (Fig. 13.3). This reflects challenges in HCV case finding, with engagement in treatment services clearly outpacing the rate of new diagnosis.

With regard to treatment outcomes, SVR rate among persons starting SOF-based regimen was 82.1%, persons failing on SOF were retreated with LDV/SOF achieving 99.2% cure rates, and persons receiving LDV/SOF as initial treatment reached SVR of 98.4%. High overall cure rates were achieved in all patients with and without advanced fibrosis (97.3% and 98.7%, respectively, Fig. 13.4). Overall SVR rates did not differ by genotype—98.5% in genotype 1, 98.3% in genotype 2, and 97.7% in genotype 3 (Fig. 13.4). The most importantly, high cure rates have been achieved without newer generation DAAs and with only LDV/SOF with or without ribavirin.

High cure rate in genotype 1 patients in Georgian cohort is in line with previous findings from clinical trials and real-life studies demonstrating similar effectiveness of LDV/SOF [20–23].

LDV/SOF in combination with ribavirin proved to be highly effective in genotype 2 and 3 patients and can be considered as pangenotypic combination at least in Georgian settings. SVR rates shown in elimination program are comparable or even

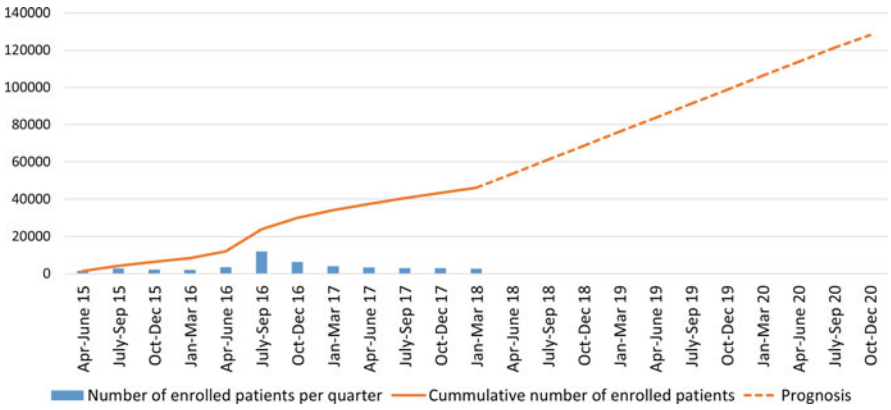


Fig. 13.3 Enrollment in HCV treatment

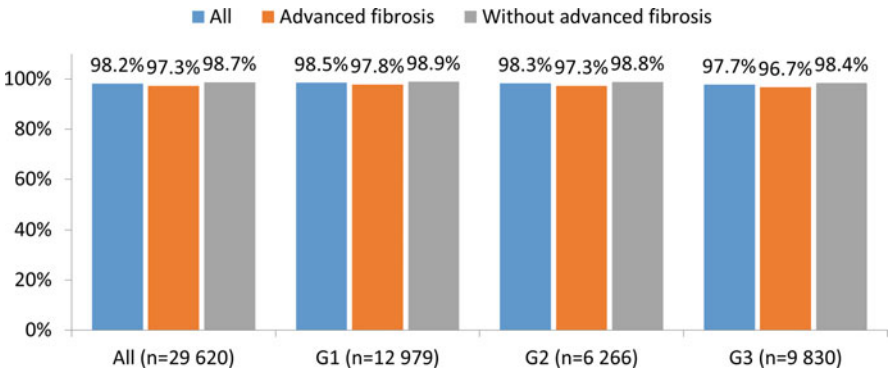


Fig. 13.4 HCV treatment outcomes by genotype and liver damage status among persons assessed for SVR, April 2015–March 2018

higher than those achieved with newer generation DAAs [24, 25]. Over 98% effectiveness of LDV/SOF in genotype 2 patients can be explained by high prevalence of RF_2k/1b recombinant form in Georgia, which has been shown to respond well to genotype 1 specific treatment options including LDV/SOF [26, 27]. Impressive results were obtained in genotype 3 patients with 97.7% SVR rate. International experience of using LDV/SOF in genotype 3 is very limited, and in the few published studies, SVR ranged between 78% and 91%, which is lower than Georgian experience [28–30].

13.4 Beyond Cascade

Georgia's elimination program has made progress in all directions of the strategic plan of action.

- *Advocacy and awareness*: Massive awareness-raising campaign has been conducting utilizing variety of media strategies (TV ads, social media ads, internet platform, etc.), short text messaging, and distribution of public education materials. Special attention has been paid to fighting stigma through engaging people living with diseases and empowering local communities [31].
- *Prevent HCV transmission*: Primary HCV prevention is one of the major activities of the national strategy. This includes harm reduction services for people who inject drugs (PWID) such as needle/syringe exchange programs and opioid substitution treatment. Available data shows that 61% of estimated number of PWID had been reached with any prevention services and 48% had been screened for HCV infection [31]. Serious efforts had been made toward implementing infection control and prevention monitoring and evaluation in medical and non-medical facilities, as well as enhancing quality control mechanisms in blood banks.
- *Improve HCV laboratory diagnostics*: Essential steps toward improving laboratory diagnostics were implemented, including approval of regulatory documents for licensing laboratory service providers and implementation of national external quality assurance program [31].
- *Surveillance*: Monitoring progress toward HCV elimination requires a well-functioning surveillance system, and efforts are made to improve system's capacity to monitor/assess the burden and risk factors for HCV infection in the country. Special study to characterize the burden of HCV-associated hepatocellular carcinoma in Georgia is underway [31].

13.5 Achieving the Goal of Elimination

Georgian hepatitis C elimination program has made substantial progress since its initiation. Over the first 3 years, more than 48,000 persons were diagnosed, and over 45,000 of them initiated treatment achieving cure in 98.2% of those assessed for SVR. Mathematical modeling study showed that these efforts already averted 2500 HCV-related deaths and 5200 new HCV infections [32].

Along with accomplishments, formidable challenges remain, and first and foremost, this relates to HCV case finding. Most people living with HCV in Georgia still remain undiagnosed representing major obstacle for meeting 90-95-95 targets. In response, Georgia is ramping up screening services along with expanding access to treatment through decentralization and integration in primary healthcare and harm reduction services. This is key for securing access to services for all and particularly for those vulnerable, such as people who inject drug.

The important feature of Georgia's elimination program is that it not only hinges on seek, test, and treat strategy but also proactively supports primary prevention through better infection control practices, blood safety, and harm reduction. Such comprehensive approach puts the country on the right path to elimination goal. Continued governmental commitment, together with active engagement from civil society and productive international partnership, provides strong basis for sealing the

success. Georgia's hepatitis C elimination program will further evolve as innovative screening strategies, diagnostics, and prevention and treatment options are implemented, providing valuable lessons for the world [33].

References

1. Baliashvili D, et al. Prevalence and genotype distribution of heaptitis C virus in Georgia: a 2015 nationawide population-based survey. J Hepatol. 2017;66:S277. https://doi.org/10.1016/S0168-8278(17)30870-X.
2. Gower E, Estes C, Blach S, Razavi-Shearer K, Razavi H. Global epidemiology and genotype distribution of the hepatitis C virus infection. J Hepatol. 2014;61:S45–57. https://doi.org/10.1016/j.jhep.2014.07.027.
3. Stvilia K, Tsertsvadze T, Sharvadze L, Aladashvili M, Rio C, Kuniholm MH, Nelson KE. Prevalence of hepatitis C, HIV, and risk behaviors for blood-borne infections: a population-based survey of the adult population of T'bilisi, Republic of Georgia. J Urban Health. 2006;83:289–98. https://doi.org/10.1007/s11524-006-9032-y.
4. Shapatava E, Nelson KE, Tsertsvadze T, del Rio C. Risk behaviors and HIV, hepatitis B, and hepatitis C seroprevalence among injection drug users in Georgia. Drug Alcohol Depend. 2006;82(Suppl 1):S35–8.
5. Chikovani I, Shengelia N, Sulaberidze L, Sirbiladze T, Tavzarashvili L. HIV risk and prevention behaviors among People Who Inject Drugs in seven cities of Georgia. Tbilisi: Curatio International Foundation, Bemoni Public Union; 2017.
6. Tsereteli N, Chikovani I, Shengelia N, Sulaberidze L. HIV risk and prevention behavior among Men who have Sex with Men in Tbilisi and Batumi, Georgia. Tbilisi: Curatio International Foundation, Center for Information and Counselling on Reproductive Health–Tanadgoma; 2015.
7. Chkhartishvili N, Abutidze A, Bolokadze N, Chokoshvili O, Dvali N, Sharvadze L, Tsertsvadze T. Hepatitis C care cascade for people living with HIV in the country of Georgia. J Int AIDS Soc. 2017;20:11–2.
8. Lomtadze N, et al. Hepatitis C virus co-infection increases the risk of anti-tuberculosis drug-induced hepatotoxicity among patients with pulmonary tuberculosis. PLoS One. 2013;8:e83892. https://doi.org/10.1371/journal.pone.0083892.
9. Butsashvili M, Kamkamidze G, Kajaia M, Morse DL, Triner W, DeHovitz J, McNutt LA. Occupational exposure to body fluids among health care workers in Georgia. Occup Med. 2012;62:620–6. https://doi.org/10.1093/occmed/kqs121.
10. Karchava M, Sharvadze L, Gatserelia L, Badridze N, Tsertsvadze T. Prevailing HCV genotypes and subtypes among hiv infected patients in Georgia. Georgian Med News. 2009;177:51–5.
11. Sharvadze L, Nelson KE, Imnadze P, Karchava M, Tsertsvadze T. Prevalence of HCV and genotypes distribution in general population of Georgia. Georgian Med News. 2008;165:71–7.
12. Karchava M, et al. High incidence of the hepatitis C virus recombinant 2k/1b in Georgia: recommendations for testing and treatment. Hepatol Res. 2015;45:1292–8. https://doi.org/10.1111/hepr.12505.
13. Zakalashvili M, et al. Identification of hepatitis C virus 2k/1b intergenotypic recombinants in Georgia. Liver Int. 2018;38:451–7. https://doi.org/10.1111/liv.13540.
14. Kalinina O, Norder H, Mukomolov S, Magnius LO. A natural intergenotypic recombinant of hepatitis C virus identified in St. Petersburg. J Virol. 2002;76:4034–43.
15. Gvinjilia L, et al. National progress toward hepatitis C elimination—Georgia, 2015–2016. MMWR Morb Mortal Wkly Rep. 2016;65:1132–5. https://doi.org/10.15585/mmwr.mm6541a2.
16. Mitruka K, et al. Launch of a Nationwide Hepatitis C elimination program—Georgia, April 2015. MMWR Morb Mortal Wkly Rep. 2015;64:753–7.

17. Dowdle WR. The principles of disease elimination and eradication. Bull World Health Organ. 1998;76(Suppl 2):22–5.
18. Ministry of Labour Health and Social Affairs of Georgia. Strategic plan for the elimination of hepatitis C Virus in Georgia, 2016–2020. Tbilisi: Ministry of Labour, Health and Social Affairs of Georgia; 2016. http://www.moh.gov.ge/uploads/files/2017/akordeoni/failebi/Georgia_HCV_Elimination_Strategy_2016-2020.pdf.
19. Nasrullah M, et al. The role of screening and treatment in national progress toward hepatitis C elimination—Georgia, 2015–2016. MMWR Morb Mortal Wkly Rep. 2017;66:773–6. https://doi.org/10.15585/mmwr.mm6629a2.
20. Afdhal N, et al. Ledipasvir and sofosbuvir for previously treated HCV genotype 1 infection. N Engl J Med. 2014;370:1483–93. https://doi.org/10.1056/NEJMoa1316366.
21. Afdhal N, et al. Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. N Engl J Med. 2014;370:1889–98. https://doi.org/10.1056/NEJMoa1402454.
22. Lim JK, et al. Safety and effectiveness of ledipasvir and sofosbuvir, with or without ribavirin, in treatment-experienced patients with genotype 1 hepatitis C virus infection and cirrhosis. Clin Gastroenterol Hepatol. 2018;16:1811-1819.e4. https://doi.org/10.1016/j.cgh.2017.12.037.
23. Tapper EB, et al. Real-world effectiveness for 12 weeks of ledipasvir-sofosbuvir for genotype 1 hepatitis C: the Trio Health study. J Viral Hepat. 2017;24:22–7. https://doi.org/10.1111/jvh.12611.
24. Foster GR, et al. Sofosbuvir and velpatasvir for HCV genotype 2 and 3 infection. N Engl J Med. 2015;373:2608–17. https://doi.org/10.1056/NEJMoa1512612.
25. Zeuzem S, et al. Glecaprevir-pibrentasvir for 8 or 12 weeks in HCV genotype 1 or 3 infection. N Engl J Med. 2018;378:354–69. https://doi.org/10.1056/NEJMoa1702417.
26. Hedskog C, et al. Characterization of hepatitis C virus intergenotypic recombinant strains and associated virological response to sofosbuvir/ribavirin. Hepatology. 2015;61:471–80. https://doi.org/10.1002/hep.27361.
27. Karchava M, et al. Impact of hepatitis C virus recombinant form RF1_2k/1b on treatment outcomes within the Georgian national hepatitis C elimination program. Hepatol Res. 2018;48:36–44. https://doi.org/10.1111/hepr.12890.
28. Dalgard O, et al. Sofosbuvir based treatment of chronic hepatitis C genotype 3 infections-A Scandinavian real-life study. PLoS One. 2017;12:e0179764. https://doi.org/10.1371/journal.pone.0179764PONE-D-17-10021.
29. Gane EJ, Hyland RH, An D, Svarovskaia E, Pang PS, Brainard D, Stedman CA. Efficacy of ledipasvir and sofosbuvir, with or without ribavirin, for 12 weeks in patients with HCV genotype 3 or 6 infection. Gastroenterology. 2015;149:1454–1461 e1451. https://doi.org/10.1053/j.gastro.2015.07.063S0016-5085(15)01096-3.
30. Ioannou GN, et al. Effectiveness of sofosbuvir, ledipasvir/sofosbuvir, or paritaprevir/ritonavir/ombitasvir and dasabuvir regimens for treatment of patients with hepatitis C in the veterans affairs National Health Care System. Gastroenterology. 2016;151:457–471 e455. https://doi.org/10.1053/j.gastro.2016.05.049S0016-5085(16)34575-9.
31. Ministry of Labour Health and Social Affairs of Georgia, National Center for Disease Control and Public Health, U.S. Centers for Disease Control and Prevention. 2017. National Hepatitis C Virus Elimination Progress Report, Georgia: 2015–2017. Tbilisi.
32. Walker J, et al. Projected impact and pathways to success of the hepatitis C elimination program in Georgia, 2015–2020. J Hepatol. 2017;66:S70.
33. Nasrullah M, Sergeenko D, Gamkrelidze A, Averhoff F. HCV elimination—lessons learned from a small Eurasian country. Georgia Nat Rev Gastroenterol Hepatol. 2017;14:447–8. https://doi.org/10.1038/nrgastro.2017.100nrgastro.2017.100.

Micro-elimination: A Key Component of Global Hepatitis C Elimination

14

Jeffrey V. Lazarus, Juan M. Pericàs, and Kristina L. Hetherington

14.1 Introduction

14.1.1 General Framework of the WHO Viral Hepatitis Strategy

As presented in Chap. 1, in 2016 the World Health Organization (WHO) adopted the *Global Health Sector Strategy on Viral Hepatitis 2016–2021* [1], to eliminate viral hepatitis as a public health threat by 2030, expanding on target 3.3 of the United Nations' Sustainable Development Goals (SDGs) [2]: to "combat viral hepatitis" [3]. The strategy relies on five strategic directions, a set of priority actions for both member states and WHO itself, and three frameworks for action (Box 14.1), including universal health coverage (UHC) as the overarching framework, a public health approach, and the concept of continuum of services.

Box 14.1 Pillars of the WHO *Global Health Sector Strategy on Viral Hepatitis 2016–2021* [1]

Strategic directions:
1. Information for focused action (know your epidemic and response)
2. Interventions for impact (covering the range of services needed)
3. Delivering for equity (covering the populations in need of services)
4. Financing for sustainability (covering the financial costs of services)
5. Innovation for acceleration (looking towards the future)
Frameworks for action:
1. Universal health coverage

(continued)

J. V. Lazarus (✉) · J. M. Pericàs · K. L. Hetherington
Barcelona Institute for Global Health (ISGlobal), Hospital Clínic, University of Barcelona, Barcelona, Spain
e-mail: jeffrey.lazarus@isglobal.org

A. Hatzakis (ed.), *Hepatitis C: Epidemiology, Prevention and Elimination*,
https://doi.org/10.1007/978-3-030-64649-3_14

Box 14.1 (continued)

2. Continuum of services
3. Public health approach
Priority actions for countries:
1. Integrate viral hepatitis strategic information activities and indicators.
2. Assess the national hepatitis burden.
3. Monitor access to, uptake, and quality of viral hepatitis services.
Priority actions for WHO:
1. Develop and update normative guidance and tools.
2. Support countries to strengthen their health information systems and to use strategic information tools.

Source: Adapted from WHO. Global Health Sector Strategy on Viral Hepatitis 2016–2021. Geneva: WHO, 2012 [1]

Just over one year after the launch of the strategy, on World Hepatitis Day 2017, WHO reported that after reviewing national hepatitis plans from 28 countries (out of the 194 WHO member states), the majority had set national hepatitis elimination targets, and most of them had begun to develop national hepatitis plans to enable access to effective prevention, diagnosis, treatment, and care services. Further, nearly half of the 28 countries surveyed were already aiming for elimination by providing universal access to hepatitis treatment. Nevertheless, WHO noted only mixed progress: "The national response towards hepatitis elimination is gaining momentum. However, at best, one in ten people who are living with hepatitis know they are infected and can access treatment. This is unacceptable" [4].

With so few countries aiming to eliminate viral hepatitis, despite adopting the WHO strategy to do so, new thinking is needed to galvanize the response. The complexity and prospective costs associated with diagnosing and treating an entire population can be overwhelming for health authorities and other stakeholders. According to Hutin et al. [5], only 20% of individuals with hepatitis C (HCV) were aware of their status in 2015, and less than 10% of those with known infection had started treatment. Fifteen million need to be urgently diagnosed and linked to treatment to achieve WHO's interim elimination targets by 2020.

One approach to disease elimination is to segment the populations considered to be at higher risk or directly affected by viral hepatitis, in order to develop a targeted response plan. This approach, called micro-elimination [6, 7], calls for a concerted effort to eliminate hepatitis C (HCV) in the subgroups where it is prevalent (target populations). These include people who inject drugs (PWID), men who have sex with men (MSM), people living with HIV (PLHIV), prisoners, people undergoing chronic hemodialysis, and people with hemophilia among a total of 12 main populations we have identified to date (see Box 14.2).

Box 14.2 12 Target Population Candidates for an HCV Micro-elimination Approach

Children (under age 15)
Coinfected with HIV
Generational cohorts of high prevalence (born 1945–1965)
Hemodialysis patients
Hemophilia patients
Men who have sex with men
Migrants from high-prevalence countries
Patients with advanced liver disease
People who inject drugs
Prisoners
Thalassemia patients
Transplant patients

An advantage to this approach is that it is multipronged and promotes external stakeholder involvement. In countries reluctant to immediately address the epidemic as a whole, initial efforts can target those already engaged with the health system such as transplanted patients, PWID regularly attending needle and syringe programs or drug consumption rooms, or PLHIV on antiretroviral therapy. This approach, which includes many marginalized and overlapping populations such as PWID, migrants, or MSM, ensures that governments do not merely address those easiest to reach and least at risk of contributing to further transmission but, rather, the majority of those likely to be affected with a particular focus on groups known to be at high risk of further spreading the disease [7].

For micro-elimination strategies to contribute to achieving the HCV elimination targets established by the WHO, including an 80% decline in HCV infections and a 65% reduction in mortality by 2030 [1], existing frameworks and principles must be aligned. Therefore, the next section will briefly discuss the main aspects of the principles of equity, UHC, a public health approach, and the priority actions for countries that should accompany an HCV micro-elimination approach.

14.1.2 Hepatitis C Treatment Delivery and Efficacy and Policies

Given that no effective vaccine exists to prevent HCV [8] and that direct-acting antiviral (DAA) drugs have been shown to be both safe and highly effective for treating HCV [9–12] and preventing its transmission [13–15], DAAs constitute the cornerstone of HCV elimination efforts. This means that scaling up and optimizing DAA use is not only an evidence-based clinical treatment model but also a strategic public health approach.

To date, several experiences from large-scale, national-level DAA treatment initiatives have provided insight into how to eliminate HCV [16–19]. However, for these insights to lead to effective and sustainable elimination of HCV, they need to be integrated into specific and multidisciplinary models of care that not only facilitate HCV patients' diagnosis and linkage to care and health professionals' communication and coordination but also are enabled by well-designed policy evaluation and cost-effectiveness analyses [20, 21].

Further, as noted by the WHO [4], most governments are not prepared to initiate ambitious, well-designed, and comprehensively funded national initiatives for HCV elimination. A recent study on hepatitis policy information reported by patient groups showed that among the 25 European countries studied, 52% had national HCV strategies, and only 44% had HCV disease registries [22]. Not only is the cascade of care proposed by the WHO [1] being hampered at its first step, namely, accurately estimating the burden of HCV in a country, but the collection of data for core monitoring and evaluation indicators is limited [23]. Policymakers might be refraining from acting for fear of increased screening leading to a rapid increase in the number of individuals diagnosed with HCV followed by the financial burden of funding DAAs and the resulting healthcare costs related to concomitant illnesses [24, 25]. As a result, the "rhetorical gap" between policymakers' commitment to eliminate HCV by adopting the WHO strategy in 2016 and real-world policy is great in most countries. In addition to a lack of a national strategy, prescription and reimbursement restrictions are also a major barrier for access to DAAs [26–28]. Even where there is a strategy or plan in place, failure to consider the unique needs of population subgroups might lead to an increase in HCV diagnosis and treatment disparities [29]. These barriers are often even more pronounced in low-resource settings such as sub-Saharan African countries [30].

14.1.3 What Do We Mean by HCV Micro-elimination?

To eliminate HCV as a public health threat, we can rely on three fundamental pillars: a global strategy to eliminate HCV; highly efficacious biomedical tools (DAA treatment) that make it possible to obtain a sustained virologic response (SVR); and strong political will despite financial and operational challenges. Yet the scale, complexity and cost of implementation for such a strategy at a global level impede a quick win. There is no "one-size-fits-all" approach, and for countries with a heavy burden, many different testing, linkage to care, and treatment models are needed [31].

Therefore, the micro-elimination approach, breaking down national elimination goals into smaller goals focusing on individual population segments, offers a sensible way forward. Pursuing the micro-elimination of HCV means working to achieve the WHO targets in specific subpopulations and settings [7]. The micro-elimination approach encourages policymakers and other stakeholders to set pragmatic national and subnational goals, while those who are best informed about the nature of the HCV epidemic in their subpopulations can take the lead in tailoring

interventions to address specific circumstances. Defining a target population for micro-elimination makes it possible to adapt case finding, treatment settings, and surveillance techniques to be more efficient and effective.

One of the advantages of the micro-elimination strategy is that it is a response based on the unique epidemiological and social contexts of a country or subnational geographic area. Many of the target populations (see Box 14.2) are already linked to the health systems, which makes them easier to reach. It is likely that other target populations may be considered after more experience is gained from current, pioneering micro-elimination studies. Potential future subgroups could include aboriginal/indigenous peoples; persons who used or have used intranasal drugs; those lost to follow-up; those who have tattoos, piercings, or scarification (as recommended in current WHO hepatitis screening guidelines) [32]; sex workers (female or male); homeless people; veterans and military personnel; refugees living in centers or camps; and/or people living in protracted armed conflict settings where international organizations are providing aid.

The advantages of adopting a micro-elimination approach within the general strategy of HCV elimination are summarized in Box 14.3. Potential synergies between these advantages should also be considered.

Box 14.3 Potential Advantages of a Micro-elimination Approach

1. It is **adaptable**, allowing for achievable, situation-specific targets to be established within the ambitious WHO hepatitis elimination framework. This enables **realistic, tailored interventions** to be put in motion.
2. Its pragmatic perspective **encourages stakeholder consensus** and effective action.
3. The **time to achievement** of elimination goals is shorter.
4. **Costs can be contained** and more easily predicted because targeted populations are smaller and some are already connected to the health system.
5. It can encompass primary prevention and the **prevention of re-infection** in targeted populations.
6. It may generate a template, including in a small geographically defined population, which may then be **used to develop services for larger intervention programs**.

14.1.4 Historical Background of Micro-elimination

The elimination of an infection or disease as a public health challenge requires deliberate efforts, which often begin through a targeted "vertical approach" at the local level [33]. However, elimination efforts can also create health systems strengthening opportunities both at the micro- and macro-levels through the integration of services, personnel training, and improved infrastructure and disease surveillance capacity, including diagnostic laboratory services [33, 34]. Historically, a

micro-elimination approach, supported by effective micro-planning activities and improved national surveillance, has contributed to the reduction of diseases such as poliomyelitis, measles, rubella, and yaws [34–37]. Eighty percent of the world's population live in certified polio-free regions—a polio eradication milestone achieved through coordinated public health activities across different geographical levels and local contexts, supported by strong a global commitment [38]. In the realm of HIV, micro-elimination efforts targeting mother-to-child transmission have significantly reduced the number of new HIV infections among children and allowed for countries to celebrate milestones [39]. For example, in 2015, Cuba became the first country in the world to eliminate both mother-to-child transmission of HIV and syphilis through a regional micro-elimination initiative [40].

The "fast-track" strategy, especially in cities, provides an example of HIV elimination efforts at a micro-level that have started to demonstrate success [41, 42]. The success to date of Iceland's national HCV elimination campaign, for example, has prompted partners in that effort to suggest that a similar model might be effective for eliminating HCV in cities with populations that are of comparable size to Iceland's national population [17]. Such an initiative might conceivably utilize a strategy that calls for micro-elimination approaches tailored to all of the city's affected populations.

14.2 The Footprints of HCV Micro-elimination in Research and Policy

14.2.1 The HCV Micro-elimination Evidence Base

To date, there are limited studies leveraging high-quality data that clarify the feasibility of micro-elimination efforts. At national and subnational levels, pioneering studies have led to key insights to improve data collection, mostly in Europe and Australia. In Belgium, for instance, where it was observed that an increase in diagnosis and treatment was necessary to achieve WHO HCV elimination targets [43], data on HCV prevalence and fibrosis stage in most target populations were scarce and largely unreliable, and there were no data for two of the subgroups investigated [44]. The study concluded that creating a centralized HCV database as an essential component of an integrated health information system with the ability to capture, store, analyze, and report HCV-related data is needed and that in fact the approximate figures of HCV infections among some target populations would allow for easily achieving elimination in them.

At the end of 2016, the Irish Haemophilia Society declared the effective "eradication" of HCV among people with hemophilia [45]. Seemingly, small European countries that developed dedicated screening campaigns have led to supportive pre-elimination scenarios. This is the case in Iceland, Georgia, and Slovenia. In 2008, the National Health Service of Scotland launched a multiphase HCV elimination plan, and through continuous action, it is believed that elimination might soon be achieved among PWID [46, 47]. If established treatment targets are met, the

Scottish government estimates a 75% reduction in the annual number of people developing HCV-related liver failure and/or liver cancer by 2020. In the USA, Facente et al. [48] (https://www.thelancet.com/journals/langas/article/PIIS2468-1253(18)30132-8/fulltext) provided a comprehensive account of the HCV epidemic among three target populations (PWID, MSM, and transgender women) and among the general population in San Francisco. Notably, the study estimated approximately 51% more HCV seropositive cases than are included in San Francisco's HCV surveillance case registry. Two of their conclusions are central to the micro-elimination approach:

> Findings from this analysis will lead to more explicit targeting of these types of prevention and treatment efforts toward PWID, MSM, transgender women, and men overall, in addition to baby boomers; future efforts to better understand disparities related to race/ethnicity would also be useful for better targeting strategies and While the study applied data sources specific to San Francisco, the methods could be applied by any region with local data sufficient for triangulation.

(https://www.thelancet.com/journals/langas/article/PIIS2468-1253(18)30132-8/fulltext)

As examples of evidence supporting micro-elimination in specific key populations, PWID are the subgroup most often addressed. It was among PWID that HCV treatment as prevention strategy was first examined [49], the reasons to prioritize PWID in elimination efforts discussed, and the need to implement tailored models of care with multistakeholder involvement raised [50, 51]. An economic modeling study conducted in France showed that rapid diagnosis/linkage to care combined with treatment initiation at the F0 fibrosis stage and improved harm reduction services were highly effective and cost-effective [52]. Another modeling study addressing the changes needed to prevent HCV transmission among PWID in 11 European countries showed that current treatment rates, if unmodified, would only achieve prevention of transmission after 10 years in three of the settings. Importantly, the study also showed that increasing coverage of needle-syringe programs (NSP) and opioid substitution therapy (OST) to 80% among PWID significantly reduced the treatment scale-up needed to reach the prevention of HCV transmission [53].

Effective HCV management in prisons has also been proven feasible. In Spain, the JAILFREE-C study [54] tested all 436 inmates in a single prison and identified 70 HCV-positive inmates (the prevalence was 15-fold higher than in the Spanish general population). Of those, 52 who were RNA positive received treatment and all achieved a SVR. The study relied on telemedicine tools, which were well-received by inmates and provided positive outcomes in terms of treatment completion and efficacy [55] and is a model that can be extended. In Australia, Bartlett et al. [56] also eliminated HCV in a prison setting: during a 22-month period, HCV viremic prevalence dropped from 12% to 1% after initiating treatment in 119 individuals.

The elimination of HCV among PLHIV has also received extensive attention. It is believed that HCV elimination among PWID and MSM is more feasible in the syndemic context of HIV coinfection with HCV, but according to modeling studies,

it would require treatment scale-up for both target populations as well as harm reduction for HIV-positive and HIV-negative PWID and behavior risk reduction in HIV-positive MSM populations [57]. The results of a cohort-based modeling study [58] among PLHIV coinfected with HCV in France led to similar conclusions. To eliminate HCV in PLHIV where the mode of transmission was sex between men and to reduce overall underdiagnosis, treatment uptake should increase by at least twofold. Sacks-Davis et al. [59] identified seven HCV elimination initiatives and studies among HCV-HIV-coinfected populations. The studies were conducted in Australia, Canada, France, Georgia, the Netherlands, and Switzerland. The main finding was that in spite of an increase in treatment uptake in the DAA era, almost a half of all identified patients remained untreated. Thus, there is a long way to go to eliminate HCV even among well-identified target populations in high-income countries. Moreover, the long-term consequences of the liver and non-liver complications should also be taken into account even if micro-elimination is reached relatively soon. For instance, in a 2015 Spanish study [60] of 1867 PLHIV, liver cirrhosis was present in 22.9% of patients after HCV therapy. This points to the urgency to address the high percentage of patients who are diagnosed and subsequently treated late [61].

14.2.2 Official Documents That Include Aspects of Micro-elimination

References to HCV micro-elimination and mentions of target populations in relevant documents from both international and national entities are limited. Box 14.4 summarizes the mentions of elimination targets among specific populations in national and international strategic documents. The main finding is that the number of subgroups considered and the level of detail of the interventions are greater in the consulted national hepatitis plans than in documents endorsed by international organizations such as the WHO and other United Nations agencies. However, it is to be noted that most of the mentions are theoretically embedded in a general direction towards achieving the general 2030 targets and put with expressions such as "control," yet almost none are explicitly expressed as searching for elimination in particular key populations. Although some documents clearly refer to the 12 target populations and, in some cases, even go beyond (e.g., Action Plan for the WHO European Region, page 3, [65]), the absence of references of any kind to target populations other than PWID, HIV, MSM, and prisoners in almost all documents consulted is disappointing. This is understandable for initiatives launched before the WHO strategy for HCV elimination as in the case of UNAIDS Fast-Track or Spanish and Australian National Plans, but not in the other cases. Of course, there are also local particularities, such as that of Scottish plans targeting people receiving blood transfusions before 1991 instead of the 1945–1965 cohort.

Box 14.4 Mention of Elimination Targets Among Key Populations in Relevant International Strategic Documents, Guidelines, and National Hepatitis C Plans

	<15 y	HIV	'45–'65	HD	HF	TL	MSM	Migr.	ALD	PWID	Pris.	Trans
WHO [1]		×					×	×		×	×	
WHO [4, 32]		×					×	×		×	×	
UN SDGs [62]										[a]		
UNAIDS Fast-Track commitments [63]		×										
CDC [64]		×								×	×	
WHO Action Plan for the European Region [65]										×	×	
Spanish Strategic National Plan for HCV [66][b]												
Australian National Plan on HCV [67][c]												
Eliminating HCV in Scotland: A call to action [47]		×					×	×	×	×	×	

Notes: ALD advanced liver disease, *HD* hemodialysis, *HF* hemophilia, *Migr.* migrants, *Pris.* prisoners, *TL* thalassemia, *Trans* transplanted patients, <15 y, children younger than 15 years; '45–'65, individuals born between 1945 and 1965

[a] The document does not mention HCV elimination not even for PWID. Remember that Goal 3 of the SDGs refers to "combat hepatitis" [68], not to eliminate it

[b] No mention of "elimination" nor "eradication"

[c] A specific section is devoted to "priority populations," and all actions related to prevention, testing, management, awareness raising, etc. are referred to them. Elimination targets were not included at the national level, although one of the main targets was to reduce the incidence of new HCV cases by 50%. In 2016, the Region of Victoria launched a Victorian strategy to eliminate HCV by 2030 [69]

14.3 General Principles of Micro-elimination Strategies: Achieving HCV Micro-elimination Requires Ensuring Equity and Human Rights

The three pillars of a micro-elimination strategy needed to accomplish the aims of the WHO HCV elimination strategy are to adapt the theoretical and empirical principles of a comprehensive public health approach to the local setting while being able to reinforce a global perspective on elimination; to place equity as a fundamental driver of change through research, policy, and advocacy; and to conceptualize and adapt the most suitable models of care for each target population in a general health systems framework aiming at UHC within the scope of the WHO "continuum of services" for HCV.

What do we understand by a global health strategy? Why should and how does HCV micro-elimination, when focused on population subgroups and often at the subnational level, fit into a global perspective? Beyond the obvious fact that HCV micro-elimination has the potential to contribute to global HCV elimination as a public health threat by generating a positive aggregate impact at the macro level, the definition and characteristics of a global health strategy set out by Abimbola [70], which contextualize global health as a matter of equity, offer further useful insights. In this regard, micro-elimination addresses equity everywhere—in high-income countries where equity should ostensibly be less of an issue and in low- and middle-income countries (LMICs); in both cases, the same two main types of problems are faced: problems of discovery (finding technological innovations to improve global health equity, including DAAs and equitable models of care) and delivery (making innovations work in practice, entailing an approach that requires the social sciences and those familiar with governance and power issues). At the same time, drawing on the work of Dillon and Karan [71], the motivations of HCV micro-elimination can be grouped into three overarching rationales: ensuring health security, promoting economic and political development, and achieving health equity as a universal human right, although in the case of HCV micro-elimination the human rights perspective should be prioritized over all other considerations.

Equity is broadly emphasized in the WHO Global Health Sector Strategy on Viral Hepatitis, and reducing health inequalities constitutes a goal in and of itself in the SDGs ([2], GBD [72]), where it is closely related to human rights. Moreover, health equity and human rights have been addressed in other important conferences and key documents related to hepatitis, such as the National Viral Hepatitis Roundtable [73] and Human Rights Watch [74]. Meanwhile, human rights promotion and UHC are tightly linked to both the SDGs and the WHO viral hepatitis strategy. This is not by chance. It has been 50 years since the right to health was included in the Universal Declaration of Human Rights, and since then, much work has been carried out to ensure access to healthcare for all, including the International Covenant on Economic, Social and Cultural Rights (1966), the Alma Ata Conference (1978), and the Ottawa Charter (1986).

Public health policy approaches have demonstrated measurable improvements in population health. Yet, "one-size-fits-all" approaches cannot be applied universally

in public health contexts and, in some cases, can widen existing disparities. It has been argued that interventions, including policy interventions, can have the greatest impact when they target the social determinants of health [75]. Although the WHO viral hepatitis strategy is framed within the necessity of developing smart and efficient models of care within a worldwide UHC perspective and therefore highlights financing for sustainability, equity and human rights should be at the core of micro-elimination strategies, surpassing the narrow definitions of equity that parallel it with equality. This means that it is essential to avoid discrimination and ensure that marginalized groups (including many of the key populations affected by HCV) have the right to access care of at least a minimum level of quality. From an equity perspective, the "right to health" approach is based on the general principle of social justice, and experts believe it is achieved when every person has the opportunity to achieve their full potential for health [76]. This is precisely where micro-elimination connects with the global strategy on HCV elimination: the pioneering studies providing reliable data on HCV prevalence by affected subgroup, the development of adapted models of care, and the implementation of rapid test and treat schemes for target populations will augment elimination efforts in other groups. The focus on smaller populations should not detract from the broader elimination goal but instead have a galvanizing effect while it serves to avoid catastrophic costs for patients and national health systems [77].

Thus, the organization of national health systems to eliminate HCV using a national hepatitis plan that includes detailed micro-elimination strategies as the main tool should rely on crossover government actions aimed at improving social and political determinants of health and the implementation of specific tools to detect health inequalities that might arise as a consequence of the application of policies [78]. Further, these policies should also aim to overcome potential barriers such as stigma and discrimination, social beliefs on freedom and law restrictions, violent conflicts, and gender, class, or racial determinants that might hamper the target population's access to the healthcare system and specifically to the continuum of care for HCV [79–82]. Contemporary examples that are representative of the entangled threats to human rights and equity are the rise of HCV cases among vulnerable populations triggered by the opioid crisis in the USA [83]; the gaps in HCV testing and treatment uncovered by the recent influx of refugees in Europe [84]; rural cohorts in parts of Africa, some of whom were possibly infected iatrogenically during colonial era health campaigns and/or could currently be part of ongoing transmission linked to persistent failures in adherence to universal precautions and unsafe cultural practices but are currently cut off from available health services [85–87]; as well as strong correlations between incarceration, injecting drug use, and HCV, especially in LMICs, shown by evidence from reviews of data from prisons which, while sparse, indicate the need to address the fact that there are few to no policies in place to address the disease burden in this population [88, 89].

14.4 Basic Requirements to Embark on the Path of HCV Micro-elimination

To ensure that both the overall aim and specific targets of micro-elimination campaigns are achieved, policymakers, researchers, and health professionals should fill the gaps in research and policy pointed out in Sect. 14.1.2 and also take resolute steps to advance in some other key areas.

(a) *High-quality data*. Local and national authorities should engage with micro-elimination efforts by facilitating the creation of central HCV databases and surveillance systems to capture the information necessary to generate accurate estimates on HCV prevalence and other relevant epidemiological indicators among key populations. Furthermore, these information systems should allow for awareness and satisfaction campaigns, which would facilitate the collection of qualitative data that might be useful to policy evaluation and design. It is essential data are timely and reliable and indicators are comparable between different locations. Finally, the design of information systems must take into account the type of data necessary to perform mathematical modeling studies.

(b) *Health systems organization*. As stated above, there is a need for appropriate models of care that allow for simplified diagnostics and linkage to care while enhancing the cascade of care to achieve HCV elimination [90–92]. Different simplified diagnostic algorithms have been proposed, including reflex-testing (using the same blood sample to perform an HCV-RNA test after a positive HCV-Abs test, thus avoiding unnecessary visits)—a key strategy component [93]. Models of care might require a high degree of sophistication to meet all of the potential needs of key populations (e.g., NSP, OST, psychological support, etc. for PWID); however, care models should ideally aim for simplicity, by including integrated or even co-located care and comprehensive processes.

(c) *Multistakeholder commitment*. An integrated model of care aimed at key populations requires the active and structural involvement of well-trained and well-informed clinicians with interdisciplinary expertise, as well as local authorities, public health agencies, specific centers of care (e.g., hemodialysis centers, hemophilia clinics, centers for PWID support, etc.), and patient groups, among others. This constitutes a unique opportunity to increase stakeholder involvement from groups that are not typically engaged in HCV such as nephrologists, addiction specialists, and patient groups other than those traditionally addressing HCV care. In addition, this might lead to increasing early diagnosis and rapid linkage to care, thus avoiding late presentation [61]. Likewise, in more advanced and integrated models of care for HCV, prescribing DAAs is simplified once the patient has entered the cascade of care. In Australia, for example, general practitioners (GPs) can prescribe DAAs, which facilitates simple point-of-care treatment [19, 94]. The success of such strategies relies on political commitment, which should not be expected to spontaneously emerge but instead must be actively pursued. Baker et al. [95] provide insight to drive political action in the case of United Nations Decade of Action on Nutrition.

Adopting framework synthesis methods, they concluded that major drivers of political commitment are strong leadership, civil society mobilization, supportive political administrations, societal change and focusing events, cohesive and resonant framing, and robust data systems and available evidence.

(d) *Communication campaigns for the general population, key populations, and health professionals*. The current high rates of HCV underdiagnosis, late presentation and low treatment uptake, as well as low rates of implementation of specific measures within HCV plans at the national level [22] indicate that even in the settings where awareness campaigns already exist, they must be strengthened and expanded. In addition to World Hepatitis Day activities conducted globally [96], some successful examples of communication campaigns include "Know More Hepatitis" by the US Centers for Disease Control and Prevention, intended to increase HCV testing among people born between 1945 and 1965 [97]; "We need to talk about Hep C," targeting key populations in Queensland, Australia [98]; and the "Healthy Liver Campaign" in New South Wales, also in Australia [11]. Communication is the cornerstone of any public health initiative, and HCV micro-elimination is no exception. Strategic communication tools must address different target populations and a broad range of stakeholders simultaneously while avoiding the dilution of fundamental messages. Recent experience shows that for a nationwide HCV campaign to be cost-effective when targeting risk groups, it should be combined with active case finding and not initially addressed to the general population [99]. Therefore, micro-elimination communication campaigns should be coordinated with wider reaching, national-level HCV elimination campaigns while still being specifically tailored towards target populations.

(e) *Educational initiatives targeted at health professionals*. The persistent low percentages of HCV testing and the high percentages of late presentation [61, 100, 101] are only two of several indicators suggesting that HCV awareness among healthcare professionals and their active participation in addressing it is very low. Moreover, awareness-raising campaigns have shown limited impact on GPs' testing practices when they are not accompanied by educational training [102]. Educational training on HCV to improve detection and management skills should begin early, e.g., during early medical coursework, and be repeated. "Generation Tomorrow," a recent experience involving students and community peers in Baltimore, Maryland, has proven to be successful for GPs in terms of HIV and HCV education, testing, and counseling, which led to an increase in HIV and HCV testing [103].

(f) *Key stakeholder participation*. The active involvement of different civil society actors is a prerequisite to generate synergies allowing for both the design of meaningful micro-elimination plans and their effective application. Such plans include contact to key stakeholders, adequate data collection, communication and testing campaigns, linkage to care, DAA reimbursement issues and solutions, and more. Patient associations, political parties, scientific societies, and the private sector should be all involved. Academic community partnerships are useful tools to enhance HCV screening [104]. Nevertheless, the lens should

focus on creating horizontally oriented and integrative platforms of participation, avoiding top-down schemes that discourage discussion and involvement in decision-making of those less formally educated or coming from disenfranchised backgrounds.

14.5 Tools and Resources to Implement Micro-elimination Approaches

There are a wide variety of financial and human resources that can be effectively used to advance progress towards global HCV elimination [105] through micro-elimination initiatives. It is essential to utilize these resources strategically to create synergies and efficiencies. Any successful micro-elimination initiative must contain simplified algorithms reflecting knowledge of a complete map of actors and a conceptual and operational mechanism that allows collaborative networks to work together on shared priorities. The following resources and tools are also relevant:

(a) *The catalytic effect of joint initiatives led by local and national governments*. The inclusion of micro-elimination activities into national health plans facilitates coordination, durability, regular updates, and effective action on specific targets. The role of state or local governmental agencies might be key to achieving consensus between diverse and sometimes conflicting stakeholder groups, such as regional authorities, patient and at-risk group representatives, and scientific associations. Yet, there are some risks in implementing a strategy mainly driven by governmental momentum. First, it might lead to a focus on short-term results which, while beneficial to election campaigns, are frequently counterproductive to public health policy development. Also, it might impose a top-down approach, resulting in the uneven engagement of civil society partners.

(b) *Mathematical modeling*. Techniques for epidemiological modeling based on complexity approaches are a powerful tool for estimating the future evolution of the HCV epidemic, as well as to design and evaluate interventions [106]. Modeling has even greater potential for well-known, specific subpopulations. Modeling studies can provide results useful for designing programmatic interventions when epidemiological studies conveying reliable HCV prevalence and mode of transmission-related data are unavailable. Although modeling approaches cannot replace robust epidemiological tracing and investigators should be aware that modeling design influences outcomes [107], modeling may provide enough insight to advance towards micro-elimination until more reliable epidemiological data specific to the key population subgroups becomes available. Several recent studies have demonstrated the potential utility of these techniques for HCV elimination efforts, including research from the Polaris Observatory HCV Collaborators [108] that provided global HCV prevalence estimates and genotype distribution and research from Scott et al. [17] that modeled HCV elimination scenarios in Iceland following

the launch of a nationwide treatment program there. Buti et al. [109] conducted HCV elimination modeling studies in Spain, as did Rusch and colleagues in Switzerland [110].

(c) *Mixed-methods*. Strong quantitative epidemiological tools, including estimates and comparable indicators, supported by qualitative assessments, can provide valuable insight into an HCV epidemic. For micro-elimination purposes, understanding the experiences of professionals, populations at risk, and patients regarding a wide range of aspects are essential to assessing an epidemic. Recognizing the challenges encountered by health professionals in case detection, how stigma acts a barrier for consultation, or the possible economic difficulties experienced by individuals in their attempt to complete treatment are all situations that can be investigated through a qualitative approach. A recent example of this is the ongoing HepCare study, which aims to develop, implement, and evaluate interventions to improve the identification, evaluation, and treatment of HCV among PWID in five European countries through a mixed-methods protocol [111]. The modeling analyses performed by the Polaris Observatory included using a Delphi process to contextualize and validate model inputs through expert consensus. Also, the DOT-C, a cluster randomized study designed to compare a pharmacist-led pathway for HCV diagnosis and treatment with a conventional pathway in Australia, broadly relied on mixed-methods for evaluation [112]. The aforementioned "Healthy Liver Campaign" [11] in New South Wales, Australia, includes two phases of qualitative data collection among PWID, including perceptions, attitudes, motivators, and barriers towards assessment and treatment of liver disease and issues related to the "four Ps" of social marketing (Product, Price, Place, and Promotion).

(d) *Technological advances*. Healthcare and public health professionals should leverage new technological advances and innovative tools with two main goals: to simplify and enhance the HCV cascade of care for key populations, accelerating the diagnosis process, linkage to care, treatment, and post-SVR follow-up, and to obtain accurate data through comprehensive and easy-to-use informatics and virtual platforms. This needs to be accompanied by the placement of modern technological tools in strategic locations such as hemodialysis centers; harm reduction centers for PWID including NSP, OST, and drug consumption rooms; and prisons to promote and ensure testing, identification, and linkage to care. HCV reflex testing to improve linkage to care and timely treatment initiation, particularly for marginalized populations, as well as other simplified diagnostic algorithms, must be made available in multifaceted points of care [50, 51, 93]. Recently, Lamoury et al. [113] compared the sensitivity of an HCV viral load assay (both in its forms of fingerstick in blood and in plasma) with those of a real-time viral load assay performed on blood obtained by venipuncture in a sample of 223 participants enrolled in drug treatment clinics and homeless services. The fingerstick assay showed 100% sensitivity and specificity, allowing for single-visit detection of HCV RNA in just 58 min. Telemedicine has proven useful for treatment and follow-up of incarcerated patients [55], and a variety of mobile apps could be considered for awareness raising, assuring linkage to care and follow-up.

14.6 Known and Potential Barriers to HCV Micro-elimination

(a) *Late presentation of HCV*. The identification of HCV-infected patients at late stages of liver disease [61] presages a higher risk of liver and non-liver complications, but most importantly, for micro-elimination purposes, it increases the potential for transmission and impedes the achievement of SVR. Therefore, micro-elimination interventions at the key population level provide a unique opportunity to investigate HCV prevalence among some of the groups at a greater risk of late diagnosis due to stigma that can lead to discriminatory practices in the health system to detect late presenters and to dedicate efforts to treat and follow up for early detection of complications and reinfection. The late presentation of HCV poses a real challenge to any health system and needs to be carefully addressed, starting by conducting rigorous studies to more accurately determine the prevalence of both late presentation and active HCV infection among the general population and in target populations.

(b) *DAA prescription restrictions*. This issue of DAA expansion has triggered a heated dialogue between the advocates who support maintaining DAA prescription as an exclusive prerogative of specialists and those who, from a public health perspective, argue that DAAs are a public health solution that needs to be expanded through simplified algorithms in single point of care that avoid unnecessary visits and loss to follow-up. Currently, over 90% of European countries require a formal indication by a specialist to initiate DAA treatment [28]. Meanwhile, the experience in Australia, where general practitioners are able to prescribe DAAs (in consultation with an HCV specialist if inexperienced in HCV management), shows that it is not only feasible but also effective [19]. From March to December 2016, 38% of DAAs in Australia were prescribed by physicians other than HCV specialists [94]. Also, a pharmacist-led pathway proved feasible for testing and treatment in PWID receiving OST in Scotland [112]. Hence, broadening DAAs prescriptive authority to GPs and other specialists with remote support from HCV specialists is fundamental to both HCV micro-elimination and global HCV elimination. In the case of micro-elimination, specialists treating key populations such as nephrologists, addiction specialists, HIV specialists, prison physicians, and others should receive training in order to substitute for HCV specialists where they are less available.

(c) *DAA reimbursement issues*. As discussed in previous sections, any initiative aiming at increasing HCV diagnosis rates and awareness among key populations must meet three requirements: there must be sufficient treatment availability for adequate coverage, DAA indications must be expanded to include those in early stages of liver disease (F0–F1) in all countries, and DAAs must be available outside of a hospital setting. According to results of the Hep-CORE study, in 2017, 80% of patient groups from the 25 countries included in the survey reported that HCV treatment was unavailable outside of hospital settings [22].

(d) *Lack of communication*. Communication gaps may arise between national and local authorities and with the general population, between medical specialties,

between centers where key populations are seen for care, and elsewhere. Such disconnection or perpetuated misunderstanding can stem from a number of sources, including differing interests, perspectives, or immediate goals. For example, in the Hep-Nordic study, in which the implementations of policies supporting key elements of national HCV elimination strategies were investigated in Denmark, Finland, Iceland, Norway, and Sweden, responses from leading stakeholders in these countries revealed a number of discrepancies between countries' policies and stakeholders' level of awareness of them [114]. The WHO and other international organizations are seldom able to monitor and resolve communication gaps or lack of understanding at the national level, let alone at the local level. Therefore, all national hepatitis elimination plans should include a communication strategy, which preferably includes regular multistakeholder consultations, to ensure stakeholder understanding.

(e) *Identification of target populations outside the health system*. As stated in Sect. 14.1.3, one of the main advantages of a micro-elimination approach is that it can increase the focus on populations at high risk of HCV transmission. However, it does not necessarily imply that all the individuals belonging to the target populations are already identified and linked to the health system. This may be even more so the case in LMICs, which have relatively high burden of HCV but weak health systems, which means key subgroups are likely to be undefined and difficult to engage. Consequently, micro-elimination efforts should be connected to awareness and testing campaigns and also to other public health and social service initiatives to ensure inclusivity. As discussed in Sect. 14.3, to ensure equity and avoid discrimination, these efforts should be included in a general strategy towards UHC in countries where it has not been reached and to protect universal access to care in countries where UHC has been standard policy for years but impeded due to financial restraints or political decisions. For instance, in the autonomous community of Catalonia, the government approved a law in 2017 to ensure universal healthcare for migrants and other vulnerable populations after the Spanish government restricted it in 2012 following a structural healthcare system reform [115]. However, as also broadly discussed, identification of key populations requires specific and robust surveillance systems at local, national, and supranational levels. For example, although Spain has an ambitious national viral hepatitis plan, it has not been accompanied by a strong and integrated central surveillance system, and the same can be said for most other European countries and the European Centre for Disease Prevention and Control, an agency of the European Commission, and without such systems, progress towards goals is impossible to track accurately, undermining the efficacy of even a well-designed plan.

14.7 Recommendations and Conclusions

Micro-elimination faces significant challenges and barriers. Evidence for action is urgently needed to persuade national and local authorities to implement micro-elimination campaigns, in collaboration with key stakeholders that include, among other relevant measures, resolving DAA prescription and reimbursement issues and location where testing and treatment can be provided. Education and communication campaigns targeting health professionals, the micro-elimination target population subgroups, and the general population are imperative. Moreover, important research gaps, largely centered around implementation research and delivery science, such as how to implement new diagnostic tools and models of care to reach more people, how to increase the frequency of timely diagnosis, and how to best ensure follow-up care, need to be addressed. Ultimately, to be successful, micro-elimination needs to function as a template for designing more widespread initiatives that can converge to meet the global goal of eliminating viral hepatitis C as a public health threat. Some recommendations emerging since this approach was put forward in 2017 include:

(a) *Insight from pioneering studies on micro-elimination*
Although there are limited examples of ongoing or successful HCV micro-elimination efforts, examples from other disease areas provide proof of concept that similar, well-designed, targeted efforts can yield success. We propose 12 population subgroups to be considered for HCV micro-elimination and encourage stakeholders to address all subgroups simultaneously, if possible, unless financial or other reasons require prioritization. Concrete micro-elimination targets should reflect the WHO Global Health Sector Strategy on Viral Hepatitis targets, applying those global elimination goals to the smaller focus population. Such an approach will help to ensure an equitable response as HCV cannot be considered to be eliminated unless the global targets have been met in all populations.

(b) *Special importance given to data quality*
An accurate epidemiological picture of HCV epidemics in target populations requires robust surveillance and information systems in each country and at a supranational level (regional or other) to facilitate coordination and capture data for priority indicators and to make reliable estimations to inform tailored interventions.

(c) *Modeling is a powerful tool for both research and policy design*
In the absence of robust surveillance systems and comparable indicators across target populations and countries, mathematical modeling techniques allow researchers and policymakers to rely on sophisticated estimations and projections of the HCV epidemics, as well as the potential impact of interventions in target populations.

(d) *Multidisciplinary networks at local, national, and international levels*
Professionals involved in HCV elimination should create local collaborative networks with other professionals and include patient associations, affected risk populations, and the political authorities, where relevant.

We believe that micro-elimination is a promising approach to operationalize the widespread call to action for global viral hepatitis C elimination within the framework of the WHO goal of eliminating viral hepatitis as a public health threat by 2030. By combining strategic elements of the global strategy and its equity-centered public health approach with the new WHO universal health coverage initiative, launched in the context of achieving the Sustainable Development Goals, coupled with a pragmatic view that enhances multistakeholder initiatives and facilitates the development of integrated point-of-care HCV diagnosis, linkage to care, treatment, and follow-up after achieving SVR for everyone, hepatitis C can be eliminated.

References

1. World Health Organization. Global Health Sector Strategy on Viral Hepatitis 2016–2021. Geneva: WHO; 2016. http://www.who.int/hepatitis/strategy2016-2021/ghss-hep/en/. Accessed 12 Apr 2018.
2. United Nations. Sustainable development goals. 2015. https://sustainabledevelopment.un.org/?menu=1300. Accessed 14 Apr 2018.
3. Lazarus JV. Hepatitis and the sustainable development goals: time for an end run. On Health, BMC Blog Network. 28 Sep 2015. https://blogs.biomedcentral.com/on-health/2015/09/28/hepatitis-sustainable-development-goals-time-end-run/. Accessed 21 Apr 2018.
4. World Health Organization. Eliminate hepatitis. Geneva: WHO; 2017. http://www.who.int/mediacentre/news/releases/2017/eliminate-hepatitis/en/. Accessed 16 Apr 2018.
5. Hutin YF, Bulterys M, Hirnschallet G. How far are we from viral hepatitis elimination service coverage targets? J Int AIDS Soc. 2018;21(Suppl 2):e25060.
6. Lazarus JV, Wiktor S, Colombo M, et al. Micro-elimination—a path to global elimination of hepatitis C. J Hepatol. 2017;67:665–6.
7. Lazarus JV, Safreed-Harmon K, Thursz MR, et al. The micro-elimination approach to eliminating hepatitis C: strategic and operational considerations. Semin Liver Dis. 2018;38(3):181–92.
8. Liang TJ. Current progress in development of hepatitis C virus vaccines. Nat Med. 2013;19:869–78.
9. AASLD-IDSA. Recommendations for testing, managing, and treating hepatitis C. 2017. http://hcvguidelines.org/ .Accessed 24 Apr 2018.
10. Dore GJ, Feld JJ. Hepatitis C virus therapeutic development: in pursuit of "perfectovir". Clin Infect Dis. 2015;60:1829–36.
11. Dore GJ, Altice F, Litwin AH, et al. C-EDGE CO-STAR Study Group Elbasvir-Grazoprevir to treat hepatitis C virus infection in persons receiving opioid agonist therapy: a randomized trial. Ann Intern Med. 2016;165:625–34.
12. EASL. EASL Recommendations on Treatment of Hepatitis C 2016. J Hepatol. 2017;66:153–94.
13. Hajarizadeh B, Grebely J, Martinello M, et al. Hepatitis C treatment as prevention: evidence, feasibility, and challenges. Lancet Gastroenterol Hepatol. 2016;1:317–27.
14. Olafsson S, Tyrfingsson T, Runarsdottir V, et al. Treatment as prevention for hepatitis C (TraP Hep C)—a nationwide elimination programme in Iceland using direct-acting antiviral agents. J Intern Med. 2018;283:500–7.
15. Zelenev A, Li J, Mazhnaya A, et al. Hepatitis C virus treatment as prevention in an extended network of people who inject drugs in the USA: a modelling study. Lancet Infect Dis. 2018;18:215–24.
16. Brouard C, Boussac-Zarebska M, Silvain C, et al. Rapid and large-scale implementation of HCV treatment advances in France, 2007–2015. BMC Infect Dis. 2017;17:784.

17. Scott N, Ólafsson S, Gottfreðsson M, et al. Modelling the elimination of hepatitis C as a public health threat in Iceland: A goal attainable by 2020. J Hepatol. 2018;68:932–9.
18. Simmons R, Ireland G, Irving W, et al. Establishing the cascade of care for hepatitis C in England-benchmarking to monitor impact of direct acting antivirals. J Viral Hepat. 2018;25:482–90.
19. Wade AJ, McCormack A, Roder C, et al. Aiming for elimination: outcomes of a consultation pathway supporting regional general practitioners to prescribe direct acting antiviral therapy for hepatitis C. J Viral Hepat. 2018;25:1089–98. https://doi.org/10.1111/jvh.12910.
20. Saab S, Parisé H, Virabhak S, et al. Cost-effectiveness of currently recommended direct-acting antiviral treatments in patients infected with genotypes 1 or 4 hepatitis C virus in the US. J Med Econ. 2016;19:795–805.
21. Wisløff T, White R, Dalgard O, et al. Economic evaluation of direct-acting antivirals for hepatitis C in Norway. PharmacoEconomics. 2018;6:591–601.
22. Lazarus JV, Stumo SR, Harris M, et al. Hep-CORE: a cross-sectional study of the viral hepatitis policy environment reported by patient groups in 25 European countries in 2016 and 2017. J Int AIDS Soc. 2018;21(Suppl 2):e25052.
23. Duffell EF, Hedrich D, Mardh O, et al. Towards elimination of hepatitis B and C in European Union and European Economic Area countries: monitoring the World Health Organization's global health sector strategy core indicators and scaling up key interventions. Euro Surveill. 2017;22:1–5.
24. Douglass CH, Pedrana A, Lazarus JV, et al. Pathways to ensure universal and affordable access to hepatitis C treatment. BMC Med. 2018;16:175. https://doi.org/10.1186/s12916-018-1162-z.
25. Zimmermann R, Kollan C, Ingiliz P, et al. Real-world treatment for chronic hepatitis C infection in Germany: Analyses from drug prescription data, 2010–2015. J Hepatol. 2017;67:15–22.
26. Barua S, Greenwald R, Grebely J, et al. Restrictions for Medicaid reimbursement of sofosbuvir for the treatment of hepatitis C virus infection in the United States. Ann Intern Med. 2015;163:215–23.
27. Marshall AD, Saeed S, Barrett L, et al. Restrictions for reimbursement of direct-acting antiviral treatment for hepatitis C virus infection in Canada: a descriptive study. CMAJ Open. 2016;4:E605–14.
28. Marshall AD, Cunningham EB, Nielsen S, et al. Restrictions for reimbursement of interferon-free direct-acting antiviral drugs for HCV infection in Europe. Lancet Gastroenterol Hepatol. 2018;2:125–33.
29. Janjua NZ, Islam N, Wong J, et al. Shift in disparities in hepatitis C treatment from interferon to DAA era: a population-based cohort study. J Viral Hepat. 2017;24:624–30.
30. Sonderup MW, Afihene M, Ally R, et al. Hepatitis C in sub-Saharan Africa: the current status and recommendations for achieving elimination by 2030. Lancet Gastroenterol Hepatol. 2018;2:910–9.
31. Lazarus JV, et al. We know DAAs work, so now what? Simplifying models of care to enhance the hepatitis C cascade. J Intern Med. 2019; https://doi.org/10.1111/joim.12972.
32. World Health Organization. Guidelines on hepatitis B and C testing. Geneva: WHO; 2017. http://apps.who.int/iris/bitstream/handle/10665/254621/9789241549981-eng.pdf?sequence=1. Accessed 20 Apr 2018.
33. Klepac P, Funk S, Hollingsworth TD, et al. Six challenges in the eradication of infectious diseases. Epidemics. 2015;10:97–101.
34. Klepac P, Metcalf CJE, McLean A, et al. Towards the endgame and beyond: complexities and challenges for the elimination of infectious diseases. Philos Trans R Soc Lond Ser B Biol Sci. 2013;368:20120137.
35. Dabbagh A, Patel MK, Dumolard L, et al. Progress toward regional measles elimination—worldwide, 2000–2016. MMWR Morb Mortal Wkly Rep. 2017;66:1148–53.
36. Dore GJ. Striving for hepatitis C eradication or control? Lancet. 2018;3:295–7.
37. World Health Organization. Global measles and rubella strategic plan: 2012–2020. 2012. http://apps.who.int/iris/bitstream/handle/10665/44855/9789241503396_eng.pdf;

jsessionid=32E7FE77373C91D3F6D299A05ABF64C5?sequence=1. Accessed 21 Apr 2018.
38. World Health Organization. Poliomyelitis: fact sheet. Geneva: WHO; 2018. http://www.who.int/en/news-room/fact-sheets/detail/poliomyelitis Accessed 22 Apr 2018.
39. Taylor M, Newman L, Ishikawa N, et al. Elimination of mother-to-child transmission of HIV and syphilis (EMTCT): Process, progress, and program integration. PLoS Med. 2017;14:e1002329.
40. Ishikawa N, Newman L, Taylor M, et al. Elimination of mother-to-child transmission of HIV and syphilis in Cuba and Thailand. Bull World Health Organ. 2016;94:787.
41. Hoornenborg E, Achterbergh RC, van der Loeff MFS, et al. Men who have sex with men more often chose daily than event-driven use of pre-exposure prophylaxis: baseline analysis of a demonstration study in Amsterdam. J Int AIDS Soc. 2018;21:e25105.
42. Stuart RM, Fraser-Hurt N, Kerr CC, et al. The City of Johannesburg can end AIDS by 2030: modelling the impact of achieving the Fast-Track targets and what it will take to get there. J Int AIDS Soc. 2018;21:e25068. https://doi.org/10.1002/jia2.25068.
43. Bourgeois S, Blach S, Blach C, et al. Achieving WHO recommendations for Hepatitis C Virus Elimination in Belgium. Acta Gastroenterol Belg. 2016;79:222–6.
44. Bielen R, Salomonsson S, Toghanian S et al. The burden of viral hepatitis C in key subgroups in Belgium: targets for micro-elimination. Paper presented at Global Hepatitis Summit 2018. June 14–17, Toronto, Canada. 2018. Abstract 1222.
45. RTE. Hepatitis C in Irish Haemophilia Population 'Eradicated'. 2016. https://www.rte.ie/news/2016/1222/840646-health-haemophilia/. Accessed 13 Apr 2018.
46. Dillon JF. The Scottish Hepatitis C Action Plan 4.0. 2015. http://regist2.virology-education.com/2015/1euhep/11_Dillon.pdf. Accessed 13 Apr 2018.
47. Hepatitis C Trust. Eliminating Hepatitis C in Scotland: a call to action. A summary of evidence from the Hepatitis C Elimination Inquiry held by the cross-party Scottish Hepatitis C Parliamentary Champions group and The Hepatitis C Trust. 2018. http://www.hepctrust.org.uk/sites/default/files/Eliminating%20Hepatitis%20C%20FINAL.pdf. Accessed 17 Apr 2018.
48. Facente SN, Grebe E, Burk K, et al. Estimated hepatitis C prevalence and key population sizes in San Francisco: a foundation for elimination. PLoS One. 2018;13:e0195575.
49. Grebely J, Matthews GV, Lloyd AR, et al. Elimination of hepatitis C virus infection among people who inject drugs through treatment as prevention: feasibility and future requirements. Clin Infect Dis. 2013;57:1014–20.
50. Grebely J, Dore GJ, Morin S, et al. Elimination of HCV as a public health concern among people who inject drugs by 2030- What will it take to get there? J Int AIDS Soc. 2017;20:22146.
51. Grebely J, Applegate TL, Cunningham P, et al. Hepatitis C point-of-care diagnostics: in search of a single visit diagnosis. Expert Rev Mol Diagn. 2017;17:1109–15.
52. Cousien A, Tran VC, Deuffic-Burban S, et al. Effectiveness and cost-effectiveness of interventions targeting harm reduction and chronic hepatitis C cascade of care in people who inject drugs; the case of France. J Viral Hepat. 2018;25:1197–207. https://doi.org/10.1111/jvh.12919.
53. Fraser H, Martin NK, Brummer-Korvenkontio H, et al. Model projections on the impact of HCV treatment in the prevention of HCV transmission among people who inject drugs in Europe. J Hepatol. 2018;68:402–11.
54. Llerena S, Cobo C, Álvarez S et al. A program of testing and treat intended to eliminate hepatitis C in a prison: the JAILFREE-C study. The 67th Annual Meeting of the American Association for the Study of Liver Diseases: The Liver Meeting 2016. November 11–16 2016, Boston, USA. 2016. Abstract 916.
55. Soler M, Llerena S, Cobo C et al. Using telemedicine to monitor patients on treatment of HCV infection in prison. The 67th Annual Meeting of the American Association for the Study of Liver Diseases: The Liver Meeting 2016. November 11–16 2016, Boston, USA. 2016. Abstract 1916.
56. Bartlett SR, Fox P, Cabatingan H, et al. Demonstration of near-elimination of hepatitis C virus among a prison population: the Lotus Glen Correctional Centre hepatitis C treatment project. Clin Infect Dis. 2018;67:460–3. https://doi.org/10.1093/cid/ciy210.

57. Martin NK, Boerekamps A, Hill AM, et al. Is hepatitis C virus elimination possible among people living with HIV and what will it take to achieve it? J Int AIDS Soc. 2018;21(S2): e25062.
58. Virlogeux V, Zoulim F, Pugliese P, et al. Modeling HIV-HCV coinfection epidemiology in the direct-acting antiviral era: the road to elimination. BMC Med. 2017;15:217.
59. Sacks-Davis R, Doyle JS, Rauch A, et al. Linkage and retention in HCV care for HIV-infected populations: early data from the DAA era. J Int AIDS Soc. 2018;21, e25051(Suppl 2)
60. Berenguer J, Jarrín I, Pérez-Latorre L, et al. Human immunodeficiency virus/hepatitis C virus coinfection in Spain: elimination is feasible, but the burden of residual cirrhosis will be significant. Open Forum Infect Dis. 2018;5(1):ofx258. https://doi.org/10.1093/ofid/ofx258.
61. Mauss S, Pol S, Buti M, et al. Late presentation of chronic viral hepatitis for medical care: a consensus definition. BMC Med. 2017;15:92.
62. United Nations. Office on Drugs and Crime, International Network of People Who Use Drugs, Joint United Nations Programme on HIV/AIDS, United Nations Development Programme, United Nations Population Fund, World Health Organization, United States Agency for International Development. Implementing comprehensive HIV and HCV programmes with people who inject drugs: practical guidance for collaborative interventions. Vienna: United Nations Office on Drugs and Crime. 2017. http://www.unaids.org/sites/default/files/media_asset/2017_HIV-HCV-programmes-people-who-inject-drugs_en.pdf. Accessed 23 Apr 2018.
63. UNAIDS. Fast Track, ending the AIDS epidemic by 2030. 2014. http://www.unaids.org/sites/default/files/media_asset/20140925_Fast_Track_Brochure.pdf. Accessed 23 Apr 2018.
64. Centres for Disease Control and Prevention. National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention. A National Strategy for the Elimination of Hepatitis B and C. 2017. http://www.nationalacademies.org/hmd/reports/2017/national-strategy-for-the-elimination-of-hepatitis-b-and-c.aspx. Accessed 18 Apr 2018.
65. WHO Regional Office for Europe. Action plan for the health sector response to viral hepatitis in the WHO European Region. Geneva: WHO; 2016. http://www.euro.who.int/__data/assets/pdf_file/0008/315917/66wd10e_HepatitisActionPlan_160555.pdf?ua=1. Accessed 16 Apr 2018.
66. Ministerio de Sanidad, Servicios Sociales e Igualdad. Plan Estratégico para el abordaje de la hepatitis C en el Sistema Nacional de Salud. May 21 2015. https://www.msssi.gob.es/ciudadanos/enfLesiones/enfTransmisibles/docs/plan_estrategico_hepatitis_C.pdf. Accessed 17 Apr 2018.
67. Australian Government. Department of Health. Fourth National Hepatitis C Strategy 2014–2017. Commonwealth of Australia. 2014. http://www.health.gov.au/internet/main/publishing.nsf/content/A68444CDED77B3A9CA257BF0001CFD80/$File/Hep-C-Strategy2014-v3.pdf. Accessed 22 Apr 2018.
68. United Nations. Transforming our World (The 2030 Agenda for Sustainable Development). 2015. https://sustainabledevelopment.un.org/post2015/transformingourworld. Accessed 23 Apr 2018.
69. Victoria State Government. Department of Health and Human Services. Victorian hepatitis C strategy 2016–2020. 2016. https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=2&ved=0ahUKEwjIud3j09DaAhWH0RQKHaQYB4sQFggvMAE&url=https%3A%2F%2Fwww2.health.vic.gov.au%2FApi%2Fdownloadmedia%2F%257B0FD1EF0A-3178-4C67-BF98-11A1C6F04425%257D&usg=AOvVaw0p_by0JRtuZWmxnZI6uBzE. Accessed 20 Apr 2018.
70. Abimbola S. On the meaning of global health and the role of global health journals. Int Health. 2018;10:63–5.
71. Dillon RS, Karan A. The blind men and the elephant—aligning efforts in Global Health. N Engl J Med. 2018;378:1374–5.
72. Collaborators GBD. Measuring progress and projecting attainment of the health-related sustainable development goals in 188 countries: an analysis from the Global Burden of Disease Study 2016. Lancet. 2017;390:1423–59.
73. National Viral Hepatitis Roundtable: Health Equity. 2017. http://nvhr.org/policy/health-equity. Accessed 10 Apr 2018.

74. Human Rights Watch: Hepatitis C. 2018. https://www.hrw.org/tag/hepatitis-c. Accessed 17 Apr 2018.
75. Hall M, Graffunder C, Metzler M. Policy approaches to advancing health equity. J Public Health Manag Pract. 2016;22:S50–9.
76. Sen A. Why health equity? Health Econ. 2002;11:659–66.
77. Lazarus JV, Pericàs JM, Colombo M, et al. Viral hepatitis: "E" is for equitable elimination. J Hepatol. 2018;69:P762–4.
78. World Health Organization. Closing the health equity gap. Policy options and opportunities for action. 2013. http://apps.who.int/iris/bitstream/handle/10665/78335/9789241505178_eng.pdf?sequence=1&isAllowed=y. Accessed 21 Apr 2018.
79. Crofts N, Louie R, Loff B. The next plague: stigmatization and discrimination related to hepatitis C virus infection in Australia. Health Hum Rights. 1997;2:87–97.
80. Grillon C, Krishtel PR, Mellouk O, et al. Treatment advocate tactics to expand access to antiviral therapy for HIV and viral hepatitis C in low- to high-income settings: making sure no one is left behind. J Int AIDS Soc. 2018;21(Suppl 2):e25060.
81. Sander G, Lines R. HIV, hepatitis C, TB, harm reduction, and persons deprived of liberty: what standards does international human rights law establish? Health Hum Rights. 2016;18:171–82.
82. Wolfe D, Luhmann N, Harris M, et al. Human rights and access to hepatitis C treatment for people who inject drugs. Int J Drug Policy. 2015;26:1072–80.
83. Zibbell JE, Asher AK, Patel RC, et al. Increases in acute hepatitis C virus infection related to a growing opioid epidemic and associated injection drug use, United States, 2004 to 2014. Am J Public Health. 2018;108:175–81.
84. Feld JJ. Extending a helping hand: addressing hepatitis C in economic migrants and refugees. Ann Hepatol. 2018;17:8–10.
85. Layden JE, Phillips R, Opare-Sem O, et al. Hepatitis C in sub-Saharan Africa: urgent need for attention. Open Forum Infect Dis. 2014;1:ofu065.
86. Njouom R, Pasquier C, Ayouba A, et al. High rate of hepatitis C virus infection and predominance of genotype 4 among elderly inhabitants of a remote village of the rain forest of South Cameroon. J Med Virol. 2003;71:219–25.
87. Njouom R, Nerrienet E, Dubois M, et al. The hepatitis C virus epidemic in Cameroon: genetic evidence for rapid transmission between 1920 and 1960. Infect Genet Evol. 2007;7:361–7.
88. Dolan K, Wirtz AL, Moazen B, et al. Global burden of HIV, viral hepatitis, and tuberculosis in prisoners and detainees. Lancet. 2016;388:1089–102.
89. Heijnen M, Mumtaz GR, Abu-Raddad LJ. Status of HIV and hepatitis C virus infections among prisoners in the Middle East and North Africa: review and synthesis. J Inter AIDS Soc. 2016;19:20873.
90. Boston Consulting Group. Road to elimination: barriers and best practices in hepatitis C management. Overview of the status of HCV care in Europe and Australia. 2017. http://image-src.bcg.com/Images/BCG-Road-to-Elimination_tcm104-166034.pdf. Accessed 24 Apr 2018.
91. Scott N, Doyle JS, Wilson DP, et al. Reaching hepatitis C virus elimination targets requires health system interventions to enhance the care cascade. Int J Drug Policy. 2017;47:107–16.
92. Zhou K, Fitzpatrick T, Walsh N, et al. Interventions to optimise the care continuum for chronic viral hepatitis: a systematic review and meta-analyses. Lancet Infect Dis. 2016;16:1409–22.
93. Fourati S, Feld JJ, Chevaliez S, et al. Approaches for simplified HCV diagnostic algorithms. J Int AIDS Soc. 2018;21(Suppl 2):e25058.
94. Hajarizadeh B, Grebely J, Matthews GV, et al. Uptake of direct-acting antiviral treatment for chronic hepatitis C in Australia. J Viral Hepat. 2018;25:640–8.
95. Baker P, Hawkes C, Wingrove K, et al. What drives political commitment for nutrition? A review and framework synthesis to inform the United Nations Decade of Action on Nutrition. BMJ Glob Health. 2018;3:e000485.
96. World Hepatitis Alliance. World Hepatitis Day 2018: eliminate hepatitis. 2018. http://www.worldhepatitisalliance.org/world-hepatitis-day/world-hepatitis-day-2018. Accessed 25 Apr 2018.

97. Jorgensen C, Carnes CA, Downs A. Know more hepatitis: CDC's National education campaign to increase hepatitis C testing among people born between 1945 and 1965. Public Health Rep. 2016;131(Suppl 2):29–34.
98. Queensland Health. Hepatitis C campaign. 2017. https://www.health.qld.gov.au/news-alerts/campaigns/diseases/hepatitis-c. Accessed 22 Apr 2018.
99. Helsper CW, Janssen MP, van Essen GA, et al. Effectiveness and cost-effectiveness of nationwide campaigns for awareness and case finding of hepatitis C targeted at people who inject drugs and the general population in the Netherlands. Int J Drug Policy. 2017;47:117–25.
100. Buti M, Pol S, Walker M et al.. A consensus definition of late presentation of viral hepatitis for medical care. HepHIV Conference, 5–7 October 2014, Barcelona, Spain. 2014.
101. Rockstroh JK, Boesecke C, Ingiliz P et al.. Has increased rollout of direct acting antiviral therapy decreased the burden of late presentation and advanced liver disease in patients starting Hepatitis C Virus therapy in Germany? EASL International Liver Congress 2018, April 11–16 2018, Paris, France. 2018.
102. McLeod A, Cullen BL, Hutchinson SJ, et al. Limited impact of awareness-raising campaigns on hepatitis C testing practices among general practitioners. J Viral Hepat. 2017;24:944–54.
103. Irvin R, McAdams-Mahmoud A, White JJ, et al. An education and field experience program to increase detection of human immunodeficiency virus and hepatitis C virus. Prog Community Health Partnersh. 2018;12:101–9.
104. Irvin R, McAdams-Mahmoud A, Hickman D, et al. Building a community—academic partnership to enhance hepatitis C virus screening. J Community Med Health Educ. 2016;6:pii: 431.
105. Cooke G, et al. Lancet commission: accelerating the elimination of viral hepatitis. Lancet Gastroenterol Hepatol. 2019;4:135–84.
106. Marx FM, Yaesoubi R, Menzies NA, et al. Tuberculosis control interventions targeted to previously treated people in a high-incidence setting: a modelling study. Lancet Glob Health. 2018;6:e426–35.
107. Fojo AT, Kendall EA, Kasaie P, et al. Mathematical modeling of "Chronic" infectious diseases: unpacking the black box. Open Forum Infect Dis. 2017;4:ofx172.
108. Polaris Observatory HCV Collaborators. Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modelling study. Lancet Gastroenterol Hepatol. 2017;2:161–76.
109. Buti M, Calleja JL, García-Samaniego J, et al. Elimination of hepatitis C in Spain: adaptation of a mathematical model based on the public health strategic plan for addressing hepatitis C in the National Health System. Med Clin (Barc). 2017;148:277–82.
110. Rusch U, Robbins S, Razavi H et al. Micro-Elimination of Chronic Hepatitis C in Switzerland: Modelling the Swiss Hepatitis Strategy Goals in Urban and Rural Regions. EASL International Liver Congress 2018, April 11–16 2018, Paris, France. 2018.
111. Swan D, Cullen W, Macias J, et al. HepCare Europe—bridging the gap in the treatment of hepatitis C: study protocol. Expert Rev Gastroenterol Hepatol. 2018;12:303–14.
112. Radley A, Tait J, Dillon JF. DOT-C: a cluster randomised feasibility trial evaluating directly observed anti-HCV therapy in a population receiving opioid substitute therapy from community pharmacy. Int J Drug Policy. 2017;47:126–36.
113. Lamoury FMJ, Bajis S, Hajarizadeh B, et al. Evaluation of the Xpert® HCV Viral Load Fingerstick point-of-care assay. J Infect Dis. 2018;217(12):1889–96. https://doi.org/10.1093/infdis/jiy114.
114. Safreed-Harmon K, Hetherington KL, Aleman S, et al. Policy responses to hepatitis C in the Nordic countries: Gaps and discrepant reporting in the Hep-Nordic study. PLoS One. 2018;13: e0190146. https://doi.org/10.1371/journal.pone.0190146.
115. Mouzo-Quintans J. [El Parlamento blinda hoy la sanidad universal]. El Pais, June 15 2017. 2018. https://elpais.com/ccaa/2017/06/14/catalunya/1497459112_092105.html. Accessed 30 Apr 2018.