Viral Hepatitis C

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Luigi Elio Adinolfi, Emanuele Durante Mangoni, Aldo Marrone, Rosa Zampino, and Luca Rinaldi

Key Concepts

- HCV is a small blood-borne RNA virus belonging to the genus Hepacivirus in the Flaviviridae family. Currently, 7 HCV genotypes, 84 subtypes and several quasispecies are recognized.
- HCV replication occurs primarily in the liver, but the virus infects and replicates into most human cells and tissues causing local and systemic inflammation, therefore is considered a systemic infection.
- HCV causes acute and chronic liver disease, cirrhosis and hepatocellular carcinoma as well as extrahepatic manifestations (cryoglobulinemia, diabetes, atherosclerosis, and lymphoproliferative, cardiovascular, neuropsychiatric and renal diseases).
- Liver steatosis and insulin resistance are features of HCV infection that accelerate liver disease progression and the development of hepatocellular carcinoma and extrahepatic manifestations.
- Direct-acting antivirals (DAAs) have been approved for HCV infection treatment with up to 98% cure rates, so HCV is largely treatable infection. In addition, DAAs are able to improve or reverse both hepatic and extrahepatic manifestations.

17.1 Introduction

The hepatitis C virus (HCV) is endemic all over the world and is a leading cause of liver disease and liver transplantation representing a significant public health problem. HCV is transmitted parenterally through contact with contaminated blood and most patients who acquire the infection are unable to spontaneously eliminate the virus, thereby developing a chronic infection that causes liver fibrosis and often evolves into cirrhosis and hepatocellular carcinoma (HCC). Although viral replication occurs primarily in the liver, HCV is able to infect and replicate into most human cells and tissues, causing local and systemic inflammation that plays a role in a wide range of extrahepatic manifestations, including lipid and glucose metabolic imbalances. Therefore, HCV infection is considered a systemic disease. Recently, direct-acting antivirals (DAAs) have been approved to treat HCV infection, which is now a largely treatable infection. In fact, DAAs are able to achieve HCV clearance in up to 98% of cases, improving or reversing both hepatic and extrahepatic manifestations.

17.2 Virology

HCV is a small, enveloped, positive-strand RNA virus. Comparison of HCV nucleotide sequences revealed the presence of genotypes, subtypes and quasispecies.

17.2.1 Taxonomic Classification and Genotypes

HCV has unique biological characteristics, ensuring its inclusion in the new genus Hepacivirus in the Flaviviridae family [1]. Due to the wide diversity of HCV strains, we currently recognize 7 genotypes and 84 subtypes, all of which can cause acute and chronic liver disease in humans.

L. E. Adinolfi (✉) · A. Marrone · R. Zampino · L. Rinaldi
Department of Advanced Medical and Surgical Sciences, University of Campania "L. Vanvitelli", Naples, Italy
e-mail: luigielio.adinolfi@unicampania.it

E. D. Mangoni
Department of Precision Medicine, University of Campania "L. Vanvitelli", Naples, Italy

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Genotype 1 is the most widespread worldwide, followed by genotypes 3, 4 and 2. The HCV genotypes may differ from one another in 25–33% of the entire genome sequence, while the subtypes are more closely related within each genotype showing a genetic diversity of 15–25%. Furthermore, the virus exists in each host as "quasispecies" a complex of genetic variants belonging to individual subtypes with similarities of nucleotide sequences ranging from 90% to 99% [2]. This genetic diversity underlies the ability of HCV to adapt to different compartments of the host, to evade the immune response and to persist chronically. Furthermore, the genetic variability of HCV is one of the main obstacles to the development of an effective active immunization strategy. Knowledge of HCV genotypes has epidemiological, pathophysiological and therapeutic implications.

17.2.2 Viral Structure

HCV consists of approximately 9.6 kb of single-stranded RNA molecule with positive polarity acting as messenger RNA for viral protein translation. Single-stranded genomic RNA encodes an open reading frame, translated into a single polyprotein from which ten viral proteins are generated (Fig. 17.1).

The HCV structural proteins—core E1 and E2 constitute the viral nucleocapsid and the envelope. The core protein consists of a positively charged domain implicated in RNA-binding and homo-oligomerization and a hydrophobic domain, involved in membrane, endoplasmic reticulum (ER) and mitochondria association. This subunit also mediates the intracellular binding of HCV to lipid droplets, one of the most relevant biological characteristics of this pathogen. The expression of the core protein in experimental animal liver cells is associated with steatosis and neoplastic transformation. E1 and E2 are extensively glycosylated proteins incorporated in the lipid double layer of the viral envelope in the form of a heterodimer and mediate the close extracellular association of HCV particles with lipoproteins and lipids [3]. E1 and E2 also possess highly variable domains that are largely responsible for escape from the host immune response, as well as conserved regions that mediate attachment and entry of viruses into target cells. Of note, E1 and E2 contain the epitopes of anti-HCV antibodies used for serological diagnosis.

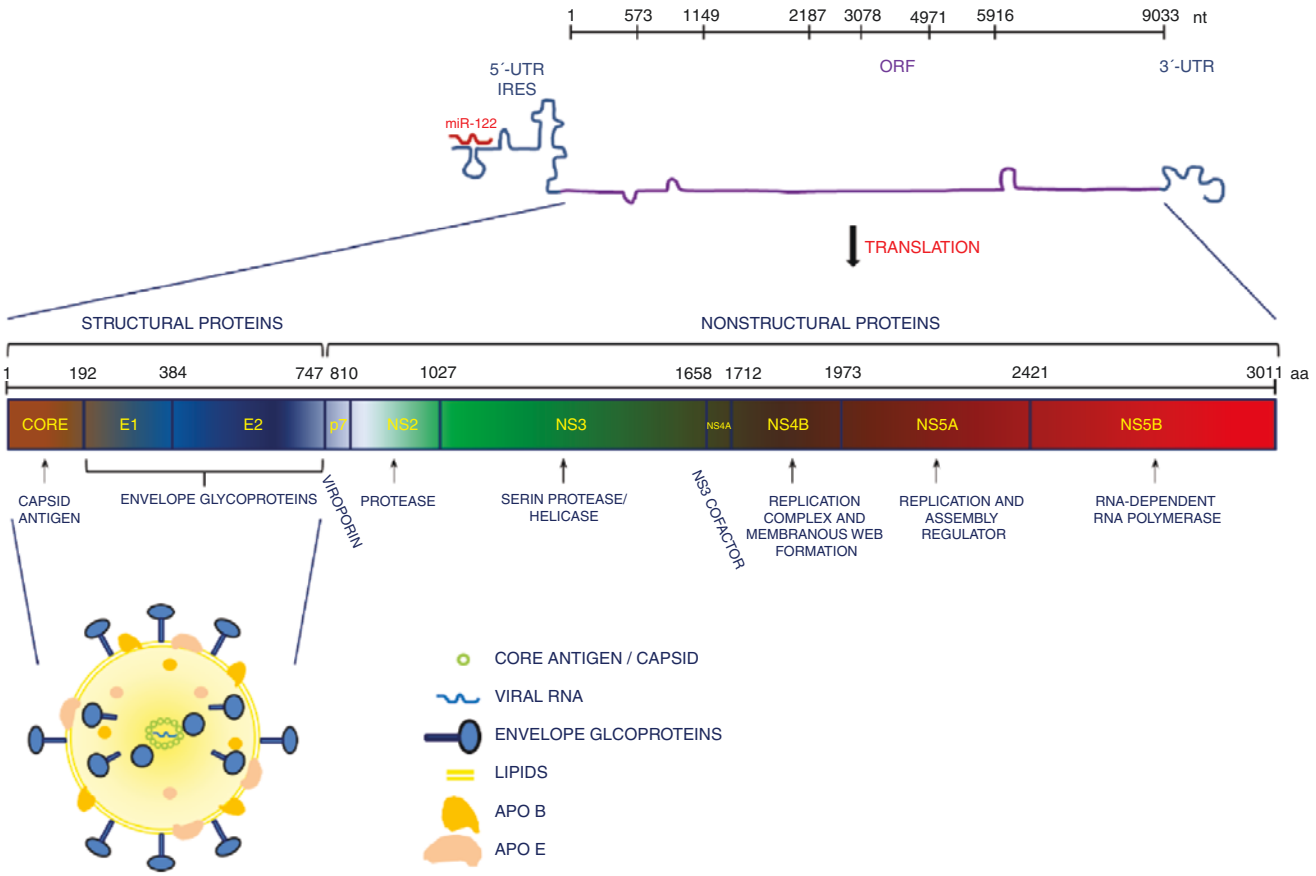

Fig. 17.1 The HCV genome

Non-structural (NS) proteins, expressed in HCV target cells, include p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B, and modulate viral replication, assembly and virulence expression. Each of the NS proteins has multiple functions. The main role in the processing of the polyprotein and in the assembly of the virus is played by the heterodimeric complex of the NS3 and NS4A proteins, consisting of a serine protease and a RNA helicase. The NS3-NS4A also breaks down the proteins of the target cells involved in antiviral signaling, hindering e.g. induction of the type I interferon pathway. NS4B is an integral membrane protein involved in the generation of membranous web. NS5A plays an important role in HCV biology by contributing to replicase formation and virion assembly. The activity of the RNA-dependent viral RNA polymerase is exploited by NS5B. Most NS proteins gather in the cytoplasm in association with a membranous network rich in vesicles to form the replicase complex, where viral RNA synthesis occurs.

17.2.3 Viral Life Cycle

Persistence is the basis of the pathology of HCV and results from both virus-induced weakening of innate and adaptive host immune responses and a regulated viral replication so as to minimize the levels of intracellular viruses and the number of actually infected hepatocytes [4].

HCV is present in the extracellular compartment in close interaction with the lipoproteins, forming the so-called lipoviroparticles (LVP). This interaction is a strategy that HCV has developed to escape antibody neutralization and to entry in the cells. Incorporated into these lipid complexes, HCV binds easily to the low-density lipoprotein receptor (LDL-R) on hepatocytes. Numerous additional receptors are also needed to mediate the binding and internalization of HCV, including CD81, the class B type I high-density lipoprotein scavenger receptor, the claudin-1 and occluding tight-junction proteins and the Niemann-Pick C1-like 1 cholesterol absorption receptor. Thus, attachment and entry of HCV are only partially mediated by a direct interaction of the envelope glycoproteins to specific host membrane protein receptors and is strongly associated with lipoprotein-mediated attachment. These multiple receptors and entry factors are exploited in an orderly manner and account for HCV infectivity in primates and tropism for hepatocytes. Initially, LVP connect to the hepatocyte surface by glycosaminoglycans, LDL-R, and class B type I scavenger receptor. The latter activates cholesterol transfer, thus freeing up virus particles from the associated lipids and allowing the interaction of CD81 with its binding sites on HCV E2. The HCV particles bound to CD81 move laterally to tight junctions, interact with claudin-1 and occludin and are endocytosed through a clathrin-dependent mechanism [5]. Within endosomes, lipid transfer activities further modify LVP and binding with CD81 primes HCV E1 and E2 to trigger fusion between the viral envelope and endosomal membrane at low pH. This probably releases the HCV genome into the cytosol, where it binds to a hepatocyte-specific microRNA, miR-122 and to cellular ribosomes through the internal ribosomal entry site. Upon release into the cytosol, the RNA genome serves as a template for both translations and replication. HCV-RNA is also used for the synthesis of negative strands, which in turn function as templates for the new HCV-RNA positive strand. The HCV genome includes highly structured non-translated 5′- and 3′-regions that flank the open reading frame, which are involved in important functions including internal ribosomal entry and translation initiation. After the formation of the NS protein, the viral replicase complex, composed of NS3 to NS5B and the genomic RNA template, begins to express its function. NS5A plays a key role. It is a multifunctional phosphoprotein associated with intracellular membranes that binds RNA and activates NS5B, the RNA-dependent RNA polymerase of HCV. It has many interactions with various cellular factors, including cyclophilin A, phosphatidylinositol-4-kinase IIIa and apolipoprotein E, which are required for HCV replication. The enzyme that catalyzes the replication of viral RNA is NS5B, which shows a typical shape of a right hand with subdomains resembling fingers, thumb and palm, designing a circular catalytic site. The negative-strand RNA synthesis begins at the 3′ end of the viral genome, and is a rate limiting step, since the positive-strand RNAs are produced in much larger quantities [6]. HCV replication is enhanced by miR-122, a liver-specific micro-RNA that also regulates the expression of fatty acid and cholesterol metabolism genes. In addition to its role in RNA replication, NS5A is also essential for the assembly of HCV and seems to interact with the core protein linked to lipid droplets. The HCV that buds into the extracellular compartment exploits the complex cellular machine that works in series with its secretory compartment. During the transport of HCV particles through the secretory pathway and the Golgi apparatus, E1 and E2 are added to the complex HCV-RNA core protein and undergo post-translational changes, including the addition of mannose and glycans. HCV particles interact with the lipids an important pathophysiological aspects of HCV infection. The available data support a connection between the maturation of HCV particles and the secretion of hepatocyte lipoproteins [7]. HCV acquires its high lipid content during hepatocyte output, in a manner very similar to the maturation of very low-density lipoprotein (VLDL) particles. This process is modulated by the microsomal triglyceride transfer protein (MTP), a large protein that actively transports the lipids into the ER lumen and in this way promotes VLDL synthesis. In the ER, VLDL synthesis results from apoB100 and lipid association. Further lipidation of VLDL precursors containing apoB occurs in

Table 17.1 Prevalence, distribution and epidemiologic and clinical features of HCV genotypes

HCV genotypes	Estimated prevalence	Prevalent geographic distribution	Epidemiologic features	Main clinical correlates
1	49%	Worldwide, especially Europe and America	Older patients, nosocomial transmission. Subtype 1a associated with IVDA[a]	Progression to cirrhosis and HCC
2	11%	Sub-Saharan Africa, East Asia	Transfusion-transmission	Cryoglobulinemia
3	18%	South Asia, Latin America, Europe	Associated with IVDA	Viral induced steatosis, younger patients
4	17%	Africa, Middle East	Health-care related acquisition	No specific finding
5	2%	Sub-Saharan Africa	Sparse data	Sparse data
6	1.5%	Southeast Asia	Sparse data	Sparse data
Mixed	1.5%	South Asia, Caribbean	Sparse data	Sparse data

[a]Intra-venous drug abuser

Golgi to form mature VLDL particles and involves apoE- and apoC-containing microsome-associated lipid droplets. The same mechanisms appear to occur during the maturation of HCV particles. Indeed, inhibition of MTP activity suppresses the production of HCV virions. Interestingly, if lipid transfer driven by MTP is slow or insufficient, apoB100 undergoes misfolding and degradation, with a consequent reduction in the production of VLDL. It can therefore be hypothesized that HCV, exploiting MTP during the production of virions in infected hepatocytes, can secondarily affect the production of VLDL. These results, on the one hand, in the common hypolipidemia observed in chronic HCV infection and, on the other, in the accumulation of lipids inside the hepatocytes, causing hepatic steatosis.

By exploiting tight-junction proteins, HCV can spread from an infected hepatocyte to an uninfected neighboring cell, thus avoiding the extracellular pathway that would most likely be prone to antibody-mediated neutralization.

17.3 Epidemiology

HCV infection is universally distributed. In 2015, a global prevalence of 1% was estimated, with 71.1 million infected subjects and 1.75 million new cases of HCV infection per year [8, 9]. The prevalence of HCV increases with age reaching a peak between 55 and 64 years. The highest prevalence of HCV was observed in the eastern Mediterranean (2.3%) and in European regions (1.5%), in the other regions the prevalence varies from 0.5% to 1% [9]. The distribution of HCV infection is different in people of diverse countries, and can be concentrated in some groups (e.g., among people who use injectable drugs) and/or in the general population. Based on the dissemination of the HCV genotype, it has been suggested that sub-Saharan Africa and Southeast Asia may be the original geographic areas of the different HCV genotypes. The spreading of HCV genotypes varies by region. The HCV genotype 1 has the highest prevalence in most countries (United States, Europe, Australia and Japan); genotype 3 is common in South Asia and genotype 4 has the highest frequency in Egypt and North Africa. Table 17.1 shows the prevalence and distribution of HCV genotypes and the main associated epidemiological and clinical features [8, 9].

17.4 Transmission

HCV is a bloodborne virus that can be transmitted even with exposure to small amounts of blood. The common circumstances of transmission are the use of illicit drug injections, unsafe injection practices and medical treatments and blood transfusions or use of unscreened blood products. Therefore, the most common practices that can transmit the virus are: (a) inject drug use by sharing syringes and needles; (b) inadequate re-use or sterilization of medical equipment in health facilities; (c) transfusion of blood or unscreened blood products. Less commonly, transmission of HCV can occur through sexual intercourse and vertical transmission from an infected mother to the child upon delivery.

Hepatitis C is not transmitted through breast milk, food, water or common relational contact of daily life, such as embracing or kissing.

17.5 Diagnosis

The HCV virologic markers consist of enzyme immunoassays (EIAs) to detect anti-HCV antibodies and HCV core antigen and nucleic acid-based molecular assays to detect and quantify HCV RNA and to define HCV genotypes.

17.5.1 Anti-HCV Antibodies

The serological diagnosis is based on the detection of anti-HCV antibodies. Currently, regardless of the viral genotype, EIA assays, which use core antigens and recombinant anti-

gens from the NS3, NS4 and NS5 regions, have a high sensitivity (97%) and specificity (99%). False positive results are possible in patients with autoimmune diseases and some infectious diseases such as mononucleosis and syphilis while false negative results may occur in immunosuppressed subjects such as those with HIV infection or hypogammaglobulinemia, in patients with solid organ transplantation and in patients on hemodialysis. The recombinant immune-blot assay, initially used as a confirmatory test, is currently considered obsolete. EIAs may be negative in the early phase of acute hepatitis C with a window period of more than 40 days. HCV-Ab positivity may persist in individuals with spontaneous or treatment-induced viral clearance [10]. Therefore, detection of anti-HCV antibodies does not document an active HCV infection that must be confirmed by the presence of serum HCV-RNA. Quantitative HCV core antigen tests are currently available. They can be a substitute test for HCV RNA where molecular biology is unavailable. The HCV core antigen may be detectable during the serological window period of the acute infection. A strong correlation between the level of HCV core antigen and viremia has been reported in patients with chronic hepatitis C [11]. However, the sensitivity of the HCV core antigen test is lower than the current HCV RNA assays.

17.5.2 Nucleic Acid Detection

The detection and quantification of HCV RNA are essential for the diagnosis of HCV infection. Real-time RT-PCR is currently the method of choice for measuring the level of HCV RNA in serum for its high and wide range of sensitivity (10–15 IU/mL up to 8 log IU/mL), the low risk of contamination and speed of execution. At least four RT-PCR assays are available considered comparable in their results. However, the accuracy of the viral load measurement may depend on the genotype**.** Currently, the Abbott Real Time HCV assay and the Roche Cobas TaqMan assay are considered to be the gold standard for the quantification of HCV RNA [12].

17.5.3 Genotyping

HCV genotyping is crucial for epidemiology and has contributed to a better understanding of different clinical manifestations. Determination of HCV genotype prior to initiation of treatment was necessary in the interferon era. However, the recent use of pan-genotypic DAAs should no longer require genotyping prior to therapy.

Commercial tests for genotyping and subtyping use genome sequencing of the core/E1, NS5B and 5′UTR regions.

17.5.4 Point of Care and Rapid Tests

The use of DAAs is increasing the interest in developing rapid and easy diagnostic tests (point of care, POCs) to achieve the WHO goal of global HCV elimination by 2030 [13]. The POC and dry blood spot (DBS) tests determine qualitative and/or quantitative viral antibodies and/or antigens. A recent rapid POC test showed a sensitivity of 98.6% and a 100% specificity in detecting HCV [14].

17.6 Clinical Manifestations

HCV infection has a wide spectrum of clinical manifestations ranging from acute and chronic hepatic and extrahepatic diseases, thus HCV is considered a multi-facet systemic disease. Figure 17.2 shows the main manifestations associated with chronic HCV infection.

Acute hepatitis C (AHC) is often asymptomatic and evolves towards chronic infection in 60–80% of cases. This in turn may slowly progress towards compensated cirrhosis and subsequently, decompensated cirrhosis. Cirrhotic patients develop HCC with an incidence of about 3% per year. Up to 70% of patients with chronic HCV infection develop extrahepatic manifestations that may be the first clinical sign of infection.

17.6.1 Acute Hepatitis

The incubation period varies between 2 weeks and 6 months, and is mostly between 6 and 9 weeks. AHC generally has mild clinical manifestations and often remains undiagnosed. In a minority of cases, symptoms such as jaundice, weakness, anorexia, malaise, dyspepsia and hepatomegaly may develop. An increase in serum alanine aminotransferase (ALT) level of at least ten times the normal value can often be observed. 20–40% of the symptomatic subjects with AHC spontaneously eliminate HCV-RNA. The average elimination time has been recently estimated at 16.5 weeks. Female sex, IL28B genotype and HCV genotype 1 are independent predictors of spontaneous clearance [15]. Fulminant hepatitis due to HCV has been reported in 5 every 1000 cases of AHC [16].

The diagnosis of AHC is often difficult due to the absence of acute serological markers. The presence of HCV RNA, anti-HCV seroconversion, the sudden increase in serum ALT values, and the recent exposure can help to perform the correct diagnosis. However, in the absence of these elements, the differential diagnosis with a reactivation of chronic hepatitis C (CHC) is difficult.

IgM anti-HCV antibodies can be detected both during the acute and chronic phases and therefore are not diagnostic.

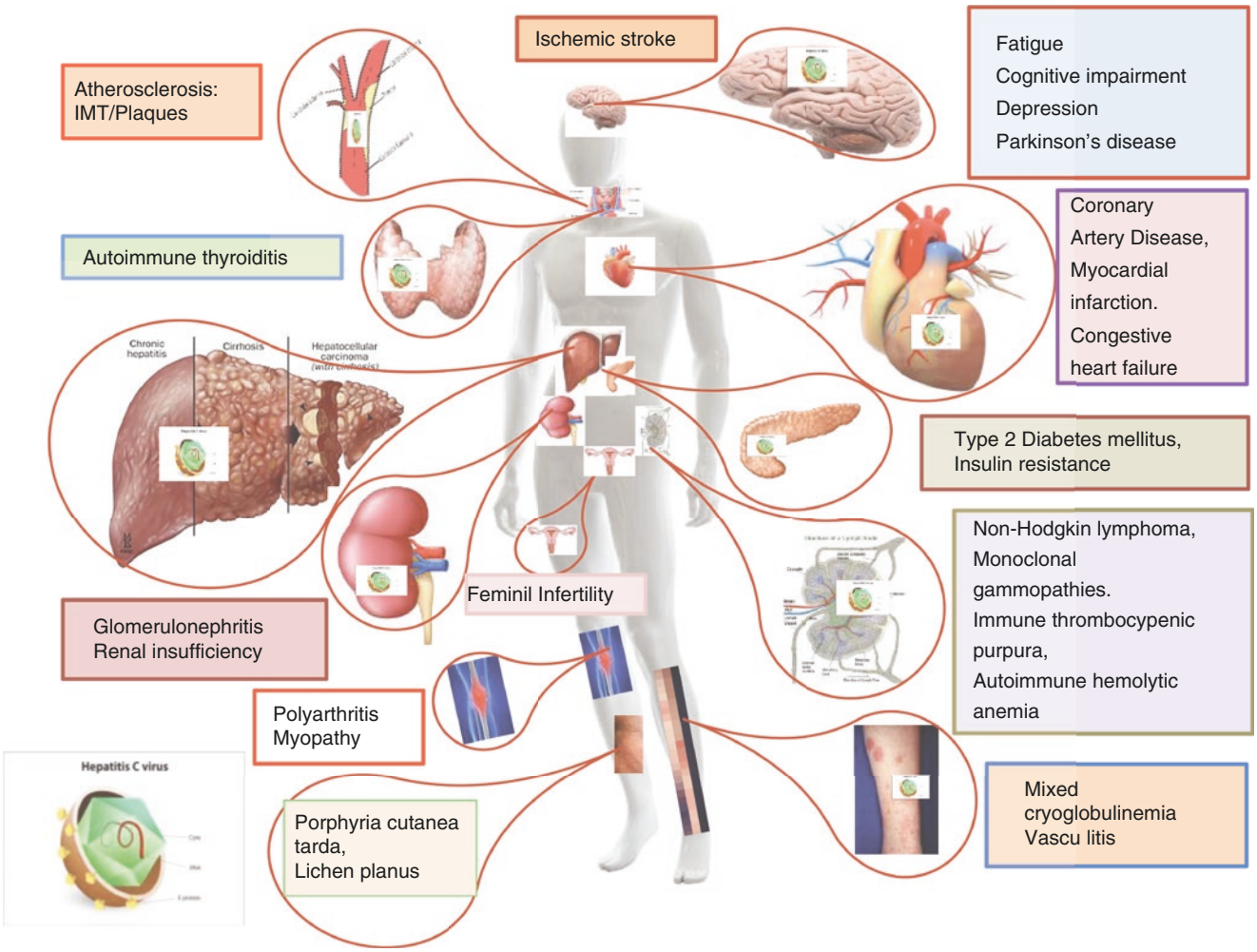

Fig. 17.2 Main hepatic and extrahepatic manifestations associated with HCV infection

Instead, the avidity test for anti-HCV IgG and IgM antibodies has recently proved to be able to make a correct diagnosis of AHC in 90% of cases [17].

17.6.2 Chronic Hepatitis

The infection is defined chronic after 6 months of HCV-RNA persistence. About 70% of infected patients show a chronic course with a slow progression of liver fibrosis toward the development of cirrhosis and its complications. CHC is generally asymptomatic or has mild and non-specific symptoms. Tiredness and malaise are the most frequent, but sometimes nausea, anorexia, myalgia, arthralgia and weight loss are possible. Hepatomegaly and splenomegaly are present in a high percentage of cases. Liver function tests are generally normal, with a mild increase in serum AST and ALT levels, although cases with persistently normal ALT are commonly observed.

Ultrasound scanning generally shows enlarged liver and/or spleen as for chronic hepatitis of other etiology, although a bright liver echo-pattern, expression of hepatic steatosis, is common in CHC. Liver biopsy and histological examination have been the gold standard in the evaluation of inflammation, fibrosis and steatosis in CHC. Currently, liver biopsy is reserved to select cases and fibrosis and disease progression are more commonly assessed using non-invasive methods including biomarkers (ARFI®, Aixplore®, Fibrotest®, FIB-4®, APRI®) and liver transient elastography (fibroscan®) [18].

17.6.3 Hepatic Steatosis

Liver steatosis is a distinctive feature of HCV infection. It is reported with an average prevalence of 55% and appears to be a final condition promoting virus survival. Viral and host factors contribute to the development of steatosis. In patients infected with HCV genotype 3, steatosis is directly

related to viral load and is considered of viral origin being defined as "viral steatosis". In contrast, in patients with non-3 genotype infection, steatosis is mainly linked to host factors such as increased body mass index (BMI), obesity, visceral obesity, insulin resistance (IR) and type 2 diabetes mellitus (T2DM), thereby being designated "metabolic steatosis" [19].

The mechanisms by which HCV induces steatosis are complex and specific for genotype 3 (impaired MTP and peroxisome proliferator-activated receptor-α (PPAR-α), increased sterol regulatory element-binding proteins (SREBPs), reduced lipoprotein export B-oxidation, increased de novo lipogenesis, downregulation of PTEN gene expression, hyper-tumor necrosis factor-α (TNF-α), and hypoadiponectinemia) and non-3 genotypes (increased BMI and IR, hypoadiponectinemia, hyper-TNF-α, increased of reactive oxygen species (ROS), of suppressor of cytokine signaling (SOCS3), of free fatty acids (FFA), of FAS activity, and impaired fatty acid oxidation and increased oxidative stress) [19]. Steatosis induces liver and systemic inflammation and oxidative stress causing a more rapid progression of hepatic fibrosis and an increased risk of developing HCC, also contributes to the development of some extrahepatic manifestations, such as diabetes, metabolic syndrome and atherosclerosis [19]. Metabolic steatosis, but not viral steatosis, reduced the IFN response rate, whereas it does not affect the response rate to DAAs.

17.6.4 Cirrhosis

The initial signs of the evolution into liver cirrhosis (compensated cirrhosis) are hardly clinically identified and can be detected by histological examination or non-invasive tests. Compensated cirrhosis may be characterized by mild changes in laboratory parameters of liver function tests, such as decreased albumin and cholinesterase levels, increased bilirubin and prothrombin time, and a variable reduction in platelet counts. However, in most cases symptoms and liver function tests are not easily differentiated from those seen in chronic hepatitis.

Decompensated cirrhosis can occur in the natural history of the disease after a variable number of years. The clinical picture and complications are like those observed in cirrhosis from other etiologies (for more details see Chap. 23). Pruritus, dryness, palmar erythema, jaundice, *faetor hepaticus*, spider nevi, petechiae, excoriation due to itching, gynecomastia and testicular atrophy can be observed. In this phase of the disease the symptoms are related to impairment of the hepatic synthetic function and to the portal hypertension. The clinical manifestations include ascites, edema of the lower limbs, jaundice, the presence of esophageal varices and their bleeding with hematemesis or melena and hepatic encephalopathy. Clinical events such as infections, hyperglycemia, renal dysfunction, cardiovascular complications and neoplasms can trigger cirrhosis decompensation events.

17.6.4.1 Ascites

Ascites, defined as the pathological accumulation of fluid in the peritoneal cavity, is the consequence of the anatomical, pathophysiological and biochemical alterations that occur in patients with cirrhosis. Three theories on the formation of ascites have been proposed: underfilling, overflow and peripheral arterial vasodilatation (see Chap. 23). The appearance of ascites is a negative prognostic factor. The presence of ascites requires a chemical-physical, microbiological and cytological evaluation. Ascites therapy (see Chap. 23) is mainly medical and consists of non-pharmacological therapy and drug therapy. Non-pharmacological therapy consists of bed rest, reduction of fluid intake and avoiding the addition of salt to the diet. Drug therapy is mainly based on the use of diuretics such as anti-aldosterone agents and loop diuretics (see Chap. 23).

Ascites can become infected with intestinal bacteria causing spontaneous bacterial peritonitis (SBP), a condition associated with a high mortality rate. Diagnostic paracentesis is always required for diagnosis of SBP. The isolation of the causative microorganism from the ascitic fluid is obtained in a small number of cases. A neutrophil count in the ascitic fluid greater than 250 cells/ml is diagnostic of SBP. Antibiotic treatment must be promptly started to reduce mortality and include third-generation cephalosporin or fluoroquinolone for community-acquired infection and a carbapenem or piperacillin-tazobactam for nosocomial infections. SBP can trigger a hepato-renal syndrome (HRS) associated with high short- and medium-term mortality. The appearance of HRS must be intensively treated using predominantly splanchnic vasoconstrictors (terlipressin) and high doses of intravenous albumin.

17.6.4.2 Esophageal Varices

Portal hypertension causes the development of esophageal varices. In patients with liver cirrhosis with liver stiffness >20 kPa, assessed by transient elastography and platelet counts <150,000/mmc, it is necessary to perform an esophagogastroduodenoscopy (EGDS) to evaluate the presence and degree of varices [20]. Patients with medium to large varices should initiate prophylaxis with non-selective beta blockers (NSBB), propranolol or nadolol or carvedilol. Nitrated and anti-aldosterone agents may be also used. All these drugs can be used alone or in combination. In cases of varices veins with a high hemorrhagic risk, endoscopic ligation should be considered (see Chap. 23). In cases of varices with a high hemorrhagic risk, endoscopic ligation should be a good option. In suspected bleeding from esophageal varices, vasoactive drugs, such as terlipressin, somatostatin, octreotide,

must be started rapidly. Furthermore, all measures must be taken to avoid hypovolemic shock. EGDS should be performed as soon as possible, in case of acute bleeding of acute esophageal variceal bleeding. In this case, ligation or sclerosis are important therapeutic options.

For secondary prevention of re-bleeding, treatment takes advantage of the NSBBs. In poorly responsive cases, a transjugular intrahepatic portosystemic shunt (TIPS) should be considered. Recently, the use of NSBB has been identified as a risk factor for portal vein thrombosis, due to the reduction of blood flow within the portal vein [21].

17.6.4.3 Hepatic Encephalopathy

Hepatic encephalopathy (HE), caused by severe liver failure and/or presence of porto-systemic shunts, is a brain alteration presenting a wide spectrum of neurological and/or psychiatric anomalies associated with a wide spectrum of clinical manifestations ranging from lowest expression that are clinical unapparent to severe clinical expression such as coma. Flapping tremor is an early sign of HE (see Chap. 63).

HE affects patients and their caregivers, because cognitive impairment makes difficult the management of these patients. The diagnosis requires the detection of signs suggestive of HE in a patient with severe liver insufficiency. The recognition of precipitating factors for HE (e.g., infection, bleeding, diuretic use, constipation, etc.) supports the diagnosis of HE and help to treat the condition.

Prevention of HE is carried out by non-absorbable disaccharides, such as lactulose or lactitol, and non-absorbable antibiotics (i.e. rifaximin at high dosage) or probiotics. Oral branched-chain amino acids can be used in chronic phase of HE, while intravenous L-ornithine L-aspartate are reserved to the acute phase.

17.7 HCC

The frequency of HCC in HCV infected patients ranges from 1% to 3% over 30 years, with an annual rate of 1–8% in the presence of cirrhosis [22]. The most important risk factors for HCC are viremia and liver steatosis [22]. Although the mechanisms involved in the development of HCC are not fully understood, a role is played by chronic inflammation, oxidative stress, insulin resistance, and endoplasmic reticulum stress. HCV structural and non-structural proteins and chronic infection are able to modulate signal pathways dysregulating cell cycle and cell metabolism, in a direct and indirect way. However, HCV does not integrate with host genome. Host genetic factors (i.e. PNPLA3 gene, CTNNB1 oncogene which encodes β-catenin protein, CDKN2A gene downregulated by HCV core protein to overcome hepatocyte senescence) can also contribute to the development of HCC [22].

Risk of HCC development is reduced, but it is not completely abolished, by antiviral treatment [23]. The presence of HCC reduces the rate of sustained virologic response (SVR) to DAAs.

Follow-up of cirrhotic patients every 6 months with US scan is fundamental for an early diagnosis of HCC lesions, even in the presence after SVR.

Patients cured for HCC with resection or ablation need to be treated with DAAs according with the recommendations for cirrhotic patients without HCC, showing similar rate of SVR than patients without HCC.

For patients with advanced HCC few therapeutic options are available. Sorafenib and Regorafenib slightly improve overall survival compared with placebo. More recently, programmed cell death protein 1 (PD1) immune check point inhibitor, as nivolumab and pembrolizumab have been evaluated in clinical trials with encouraging results in controlling small intrahepatic metastatic nodules and many other molecules are under evaluation.

17.8 Extrahepatic Manifestations

During chronic HCV infection, two-thirds of patients experienced extra-hepatic manifestations. Patients may develop one or more extrahepatic manifestations (Fig. 17.2) and these conditions are often the first and only clinical sign of infection [24]. Some of these conditions are common and well documented, while others are less frequent. Non-hepatic HCV-related conditions such as autoimmune or lymphoproliferative, cardiovascular, renal, metabolic and central nervous system diseases have been reported tightly associated with infection [24]. HCV infection was associated with a higher mortality rate for extrahepatic complications, while viral eradication significantly reduced the rate of extrahepatic deaths [25–27].

Extrahepatic manifestations may occur at any time during chronic HCV infection, therefore HCV patients should have a regular assessment for these complications during the initial visit and follow-up; conversely, patients with manifestations listed in Fig. 17.2 should be tested for HCV infection. Because of these associations, besides the liver, a thorough clinical and laboratory examination of an HCV-infected patient should cover hematologic, cardiologic, nephrologic, endocrinologic and rheumatologic signs and symptoms and a skin evaluation for findings of cryoglobulinemia, porphyria cutanea tarda and lichen planus.

17.8.1 Mixed Cryoglobulinemia

Mixed essential cryoglobulinemia (MC) is a lymphoproliferative disorder characterized by circulating serum immunocomplexes coupled to activated complement that precipitate

into small and medium-sized blood vessels. More than 90% of patients with MC are infected with HCV and about half of HCV patients have cryoglobulins [24]. HCV infection is associated with types II and III MC. The data suggest a causal association between HCV infection and MC. Predisposing factors are female sex, age, advanced liver fibrosis [24]. The diagnosis of MC can be performed by leaving the serum at 4 °C for 7 days showing a typical visible cryoprecipitate which dissolves at 37 °C. Patients have low serum C4 levels and positive serum rheumatoid factor. Most of the cases of MC are asymptomatic. Manifestation and clinical signs depend on leukocytoclastic vasculitis with palpable purpura, neurological and renal damage, and arthralgia. Purpura often involves the legs and can leave brown spots on the skin after it resolves. Vasculitis can cause ischemic necrosis and cutaneous ulceration. Vasculitis may involve the vasa nervous, more frequently of peripheral nerves of the lower limbs causing asymmetric peripheral neuropathy predominantly sensory neuropathy, although it is possible to observe sensory motor neuropathy and multiplex mononeuritis. Arthralgia and myalgia are reported in over 70% of cases and often affect the proximal interphalangeal and metacarpophalangeal joints of hands, knees and ankles [24]. Renal involvement is one of the most serious complications of cryoglobulinemia. The membranoproliferative glomerulonephritis is the typical histological lesion observed in MC. Failure to treat can cause progressive renal failure [24].

The main therapeutic approach of MC should be focused on the eradication of HCV. The clinical improvement of MC is reported in most patients who have eliminated HCV by antiviral therapy. Patients with HCV-related glomerulonephritis should be treated with DAAs, in which SVR in a high proportion of cases leads to improvement of proteinuria and even full remission of glomerulonephritis [28]. In case of non-response or with advanced conditions, corticosteroid therapy and plasmapheresis may be alternative therapeutic options. Rituximab, a monoclonal anti-CD20 antibody, which causes B-cell depletion is an effective and safe treatment for MC. Rituximab is particularly indicated in patients who do not respond to antiviral therapy and in cases of severe vasculitis [25].

17.8.2 Lymphoproliferative Disorders

HCV has been widely associated with lymphoproliferative disorders and in particular non-Hodgkin lymphoma (NHL). It has been demonstrated that HCV infection leads to a two-fold increased the risk of development of NHL. Moreover, the mortality rate for NHL was two-times higher among HCV positive patients. About 10% of long-lasting HCV infection associated with mixed cryoglobulinemia type II evolve into NHL [29]. The pathogenic mechanisms are complex and involve direct effects of HCV during viral replication within B cells, which may activate proto-oncogenes (i.e., BCL2) and/or inhibition of apoptotic factors (i.e., p53, c-Myc), and indirect mechanisms such as continuous antigen stimulation and/or genetic aberration (i.e., t(14:18) translocation).

17.8.3 Cardiovascular Manifestations

Patients with HCV infection showed an increased risk of sub-clinical atherosclerosis, peripheral artery disease, heart failure and stroke, as well as increased cardiovascular mortality [30]. Several direct and indirect mechanisms have been hypothesized by which HCV can induce or facilitate the development of atherosclerosis. HCV has been shown to live and replicate in carotid plaques, supporting the hypothesis that HCV plays a direct pro-atherogenic role by inducing arterial inflammation. In addition, HCV infection causes hepatic and systemic inflammation and structural and non-structural HCV proteins play an important role in initiating and maintaining chronic inflammation that promotes atherosclerosis development [30]. HCV can also be involved in the development of atherosclerosis through the increase of pro-atherogenic chemokine and cytokine levels, increasing levels of oxidative stress and endothelial dysfunction. HCV also interferes with glucose and lipid metabolism, leading to IR, diabetes and hepatic steatosis which are known factors that induce atherosclerosis and increase the risk of cardiovascular disease [30]. HCV induces a chronic inflammatory vessel damage and instability of plaque. Such conditions significantly increased the risk of ischemic stroke in HCV patients. HCV clearance by interferon or DAAs has been shown to improve or reverse carotid atherosclerosis and reduce both cardiovascular events and mortality [27].

17.8.4 Neurologic and Psychiatric Diseases

Chronic HCV infection is associated with neuropsychiatric disorders in up to 50% of cases. Both the central and peripheral nervous system can be involved. Neurological conditions comprise encephalopathy, myelitis, encephalomyelitis, and cognitive impairment, whereas “brain fog”, depression, anxiety, and fatigue are the main psychiatric disorders [31]. Moreover, HCV infection causes both motor and sensory peripheral neuropathy mostly associated with mixed cryoglobulinemia. The neuropsychiatric manifestations are independent of severity of the underlying chronic liver disease and hepatic encephalopathy. Direct and indirect mechanisms have been postulated [31]. The brain is a suitable site for HCV replication, in which the virus may directly exert neurotoxicity; other mechanisms proposed include the imbalance

of the metabolic pathways of infected cells, alterations in the circuits, autoimmune disorders, and cerebral or systemic inflammation. A pathogenic role for HCV is also suggested by improvement of neurological and psychiatric symptoms in patients achieving a sustained virologic response following interferon treatment [31]; however, further studies are needed to evaluate the impact of treatment with DAAs on neuropsychiatric disorders associated with HCV.

17.8.5 Endocrine Diseases

HCV infection is strictly associated with an increased prevalence of IR and T2DM. IR has been reported in up to 70% in chronic HCV infection and this prevalence is higher than that observed in HBV infection and in the general population. IR is implicated in the development of hepatic steatosis and T2DM, which is shown with a higher prevalence in HCV patients than uninfected subjects [32]. It has been estimated that up to 33% of chronic HCV infected patients have T2DM. A two- to tenfold increase of T2DM has been reported in chronic HCV infection compared to liver diseases of other etiology. In particular, the prevalence of T2DM is two- to three-times higher in HCV than HBV infection. IR and T2DM accelerate the progression of liver fibrosis, the onset of HCC and some extrahepatic manifestations such as atherosclerosis and cardiovascular diseases [33].

HCV plays a direct role in the development of IR through the core protein. HCV genotypes 1 and 4 infected patients showed the highest prevalence of IR; in these genotypes, IR correlates with HCV RNA levels. Furthermore, HCV lives and replicates within pancreatic β-cells causing distress; in addition, HCV interferes with insulin signaling pathways, with host genetic and environmental factors inducing cytokine imbalance and liver steatosis [33].

IR or T2DM significantly reduced the rate of SVR to IFN, but not to DAAs. Current data show that HCV clearance by DAAs improves or reverses IR and fasting glucose levels, reduces glycated hemoglobin levels, induces a better control of T2DM and reduces the onset of de novo IR and T2DM [26, 34].

17.9 Natural History

Acute HCV infection is self-limiting in 20–30% of cases and in the other 70–80% the infections becomes chronic. The rate of chronicity can be affected by several factors such as age at time of infection, gender, ethnicity, and the presence of jaundice at the onset. Chronic HCV infection leads to a wide range of hepatic diseases, including chronic hepatitis, cirrhosis and HCC. A number of patients with cirrhosis remain stable and well compensated for years, while others develop complications of cirrhosis particularly related to portal hypertension (esophageal varices and hemorrhage, ascites and encephalopathy) and HCC.

The risk of developing cirrhosis within 20–30 years from the infection is estimate at 20–30%, although percentages are different in relation to the studied population.

The natural history of HCV is negatively influenced by various demographic, virologic, clinical and lifestyle factors. The duration of HCV infection, the male gender and ageing are the main risk factors for the progression of liver disease to cirrhosis and HCC; other factors include HCV genotype 3 infection, host genetic polymorphisms (PNPLA3, TGFB1), hepatic steatosis, IR, T2DM, obesity, alcohol use, daily use of marijuana and viral co-infection (HBV, HIV). The appearance of decompensated cirrhosis has a negative impact on natural history of the disease. The presence of ascites is associated with a 3-year mortality rate of 50%, while in the presence of a refractory ascites 1-year survival is 50%. Chronic HCV infection causes about 400,000 deaths each year, mainly due to cirrhosis and HCC [8, 9]. In this estimate, deaths due to extrahepatic manifestations of HCV are not considered. Due to the recent introduction of DAA therapeutic regimens the natural history of HCV infection has been revolutionized. DAAs have proven to be highly effective not only in inducing the elimination of HCV, but also in improving or reversing liver injury and many of the extrahepatic manifestations associated with HCV.

17.10 Treatment

As shown in Fig. 17.3, HCV therapy has been constantly evolving, resulting in a definitive cure for all cases of hepatitis C. The IFN has been a cornerstone of therapy for more than 20 years. Currently, the IFN-free DAA regimens allow the possibility of treatment to almost all the infected population including patients with advanced stages of the disease and with severe comorbidities (e.g., renal failure) always maintaining a high efficacy and an excellent tolerability profile.

These therapeutic regimens therefore represent the ideal weapon for achieving the ambitious WHO global hepatitis C virus eradication project by 2030 [9]. However, to ensure that the eradication can be achieved it is necessary to implement screening programs to identify HCV-infected populations and that access to therapy with DAAs is made possible on a large scale [35].

17.10.1 Objectives of Therapy

The goal should be to treat all HCV-positive patients for the purpose of eliminating HCV infection in order to improve

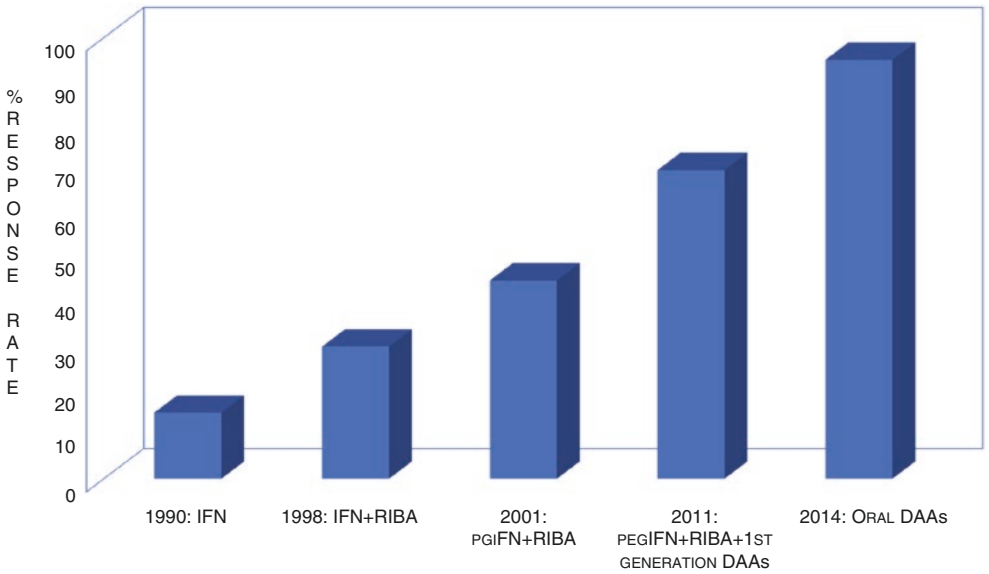

Fig. 17.3 Evolution of the treatment of chronic hepatitis C

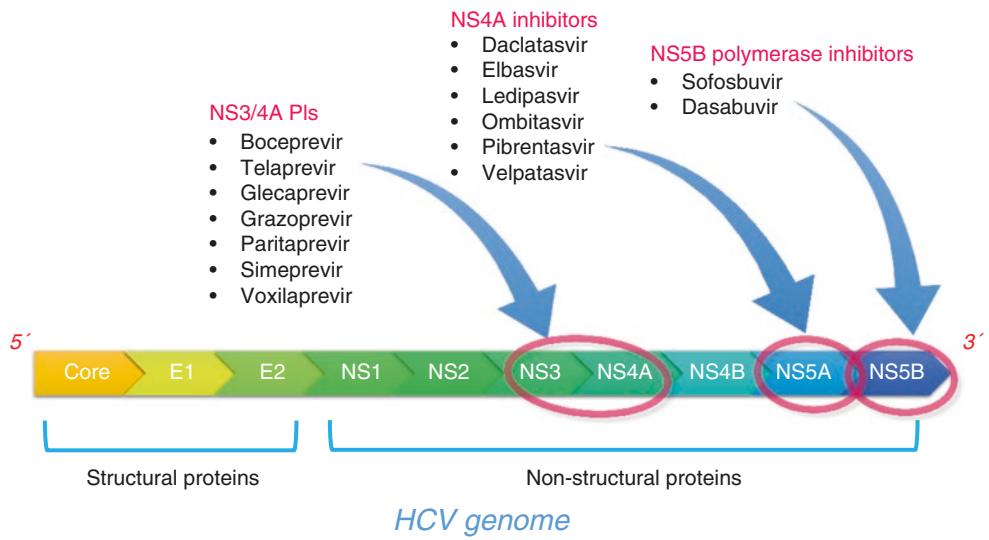

Fig. 17.4 DAAs molecular targets on HCV RNA structure

and prevent: (a) progression of liver injury and its complications; (b) extrahepatic manifestations; and (c) to reduce mortality; as well as to improve quality of life and prevent transmission of infection.

HCV RNA clearance 12 weeks after the end of DAA treatment is indicative of sustained virological response (SVR).

17.10.2 Direct Antiviral Drugs

DAAs act by inhibiting non-structural proteins and can be classified into three classes according to the target site: NS3/4A protease inhibitors (PI), NS5A inhibitors, NS5B RNA polymerase inhibitors (Fig. 17.4).

17.10.2.1 NS3/4A Protease Inhibitors

Boceprevir and Telaprevir were the first-generation drugs of this class, effective only towards genotype 1, with a low barrier to genetic resistance and therefore the need to use them in triple combination with pegIFN + RBV. For the development of resistance and for an extremely low tolerability profile, these drugs are no longer used. Second and third generation of NS3/4A inhibitors (e.g., Glecaprevir, Grazoprevir, Paritaprevir, Simeprevir, Voxilaprevir) have solved many of the problems associated with the use of first-generation drugs, showing a high barrier to genetic resistance, wide antiviral activity which include genotype 1 and 4, less significant activity on genotype 2, poorly effective on genotype 3, and few side effects. These features allow these drugs to be used in IFN-free association regimes and in a large number of patients.

17.10.2.2 NS5A Inhibitors

Inhibition of the NS5A enzyme has an important impact on viral replication at different stages of HCV life cycle. NS5A inhibitors (Daclatasvir, Elbasvir, Ledipasvir, Ombitasvir, Pibrentasvir, Velpatasvir) show a high antiviral activity towards all of the genotypes (pan-genotypic), but at the same time a relatively low genetic barrier, so it is necessary to combine these drugs with other DAAs.

17.10.2.3 NS5B RNA Polymerase Inhibitors

The NS5B protein is an RNA-dependent RNA polymerase capable of catalyzing the viral RNA synthesis and therefore represents a crucial phase of the HCV life cycle. Depending on the site of action, there are two different classes of RNA polymerase NS5B inhibitors. The nucleoside inhibitors (Sofosbuvir) act as a false polymerase substrate, which will be incorporated into the nascent RNA chain resulting in premature closure of the chain itself. Because the polymerase structure is highly conserved among all viral genotypes, Sofosbuvir has a pan-genotypic efficacy and a high genetic resistance barrier. Non-nucleoside NS5B inhibitors (Dasabuvir), on the other hand, act as allosteric inhibitors, binding outside the active site of the polymerase and causing conformational changes that inactivate the enzyme. Unlike Sofosbuvir, the range of non-nucleoside inhibitor activities is limited to genotype 1 and the genetic barrier is low.

17.10.3 DAA Therapeutic Regimens and Their Clinical Use

The use of combinations of drugs for the treatment of HCV is an inevitable strategy to prevent the onset of resistance. In fact, RNA polymerase in RNA viruses (such as HCV) is inherently error prone due to the absence of proofreading. Combined with an extremely high viral turnover, this error trend leads to a myriad of viral variants that coexist within a single host (quasispecies). Some of these variants seem intrinsically resistant to DAAs and would be rapidly selected in the case of monotherapy, thus becoming the predominant population in a short time, causing treatment to fail. Therefore, at present, a combination of two or more DAAs, with pre-established dosage is the base for the treatment of HCV infection. Therefore, each regimen requires a treatment protocol with a pre-established duration, with a selective action on specific genotypes or a pan-genotypic activity, depending on which combination of drugs has been selected (Table 17.2).

The drug combinations shown in Table 17.2 are remarkably effective, with an SVR of over 95% [36]. The duration of treatment with DAAs for HCV infection is relatively short and depending on the genotype and the absence or presence of cirrhosis and usually range from 8 to 24 weeks [36]. The duration of treatment is also influenced by the host's own parameters (e.g., stage of liver disease, naïve or experienced status for a previous IFN- or DAAs-based treatment). Advanced stages of disease (e.g., hepatic cirrhosis) and/or the status of experienced (e.g., previous treatment failure) generally require longer treatments and/or the use of RBV [36].

The tolerability profiles of second and third-generation DAAs is excellent. The most common side effects (asthenia, headache and itching) are generally mild and do not required discontinuation of treatment. Also, the presence of severe hepatic failure (Child-Pugh score B or C) or severe kidney failure (GFR <30 ml/min/1.73 m^2) cannot be considered an absolute contraindication to therapy, but influence the choice of therapeutic regimen to be adopted for a single case. For instance, Sofosbuvir is contraindicated in case of GFR <30 ml/min/1.73 m^2, whereas Glecaprevir/Pibrentasvir and Elbasvir/Grazoprevir combinations are contraindicated in case of cirrhosis in the Child-Pugh B-C score. Therefore, the high tolerability profile of DAAs and the possibility of choosing between different regimens in relation to patients' clinical condition makes these therapeutic regimens virtually possible for every HCV infected patient. To date, the only absolute contraindication is represented by the coexistence of a double organ failure, that is severe liver and kidney failure.

Table 17.2 DAA regimens approved for treatment of HCV infection in 2019

Drug	Activity	Concentration for tablet (mg)	Posology (n° of tablet/day)	Treatment duration (weeks)
Sofosbuvir	Pan-genotypic	400	One tablet	12–24 ± Ribavirin
Sofosbuvir/Velpatasvir	Pan-genotypic	400/100	One tablet	12–24 ± Ribavirin
Sofosbuvir/Velpatasvir/Voxilaprevir	Pan-genotypic	400/100/100	One tablet	12
Glecaprevir/Pibrentasvir	Pan-genotypic	100/40	Three tablets	8–12–16[a]
Grazoprevir/Elbasvir	Genotypes 1, 4	100/50	One tablet	12–16 ± Ribavirin
Paritaprevir/Ombitasvir/ Ritonavir + Dasabuvir	Genotypes 1, 4	75/12.5/50 250	Two tablets	12–24
Sofosbuvir/Ledipasvir	Genotype 1,3,4	400/90	One tablet	8–12–24 ± Ribavirin

[a]8 weeks in non-cirrhotic patients; 12 weeks in cirrhotic; 16 weeks in genotype 3

17.10.3.1 DAA Treatment and Drug-to-Drug Interaction

DAA therapy presents a challenge, the potential drug-drug interactions. The interaction risk assessment must be evaluated prior to starting therapy and before starting other medications during treatment. The DAAs pharmacological interactions are currently highly predictable and there are online websites that provide help in predicting potential drug-drug interactions (e.g., www.hep-druginteractions.org).

17.10.3.2 Post-Treatment Follow-Up

Patients with advanced fibrosis (F3) or cirrhosis who have achieved SVR should be monitored for HCC every 6 months by ultrasound. Patients with pre-treatment oesophageal varices should be periodically monitored by endoscopy.

17.11 Treatment of HCV Acute Hepatitis

AHC should be treated, similarly to those with chronic hepatitis, with a DAA regimen for 8 weeks. Considering that late recurrences have been reported, SVR should be evaluated 12- and 24-weeks post-treatment [36].

17.12 Treatment of Particular Patients with HCV

17.12.1 HBV and HIV Co-Infected

HBV-HCV coinfected patients should be treated with the same regimens used for HCV-infected patients.

In patients HIV-HCV coinfected drug-drug interaction is of particular importance, and special attention should be paid to anti-HIV drugs that are contraindicated, not recommended or that require dose adjustment with DAA regimens.

17.12.2 End-Stage Liver Disease

HCV patients with end-stage liver disease not suitable for DAA treatments, the therapeutic choice is liver transplantation. Recurrence of HCV after transplantation occurs universally, reducing the life expectancy of graft and patient survival. Post-transplant HCV recurrence should be considered as early as possible for DAA treatment.

17.12.3 Patients with Renal Insufficiency

Patients with mild to moderate renal impairment (GFR ≥30 ml/min) may be treated according to the general recommendations. Patients with GFR <30 ml/min and in haemodialysis can be treated with particular caution, sofosbuvir based-regimens should be avoided and these patients should be treated with a fixed regimen of glecaprevir/pibrentasvir for 8 or 12 weeks.

17.12.4 Non-hepatic Solid Organ Transplantation Patients

HCV patients on the waiting list for solid organ transplantation can be treated with DAAs according to the general recommendations. Similarly, organ transplant recipients should be treated considering the drug-to drug interaction.

17.12.5 Re-treatment of Non-SVR to DAA

A very small number of patients failed to achieve SVR with DAAs. In some case such failure is associated with the presence of resistance-associated substitutions (RASs) that confer reduced susceptibility to the corresponding classes of drugs. To optimize treatment, these patients must be screened for RASs before starting a new treatment.

17.13 Prevention of HCV Infection

There is currently no specific prophylaxis for HCV infection, nor is there any indication for antiviral therapies with DAAs as post-exposure prophylaxis without a documented HCV transmission [36]. Therefore, prevention is done through the correct application of the general rules of prophylaxis to prevent the spread of parenteral and sexually transmittable viruses.

The primary prevention interventions recommended by WHO are:

- hand washing and use of gloves;
- safe and appropriate use of health care injections;
- safe handling and disposal of sharps and waste;
- provision of comprehensive harm-reduction services to people who inject drugs including sterile injecting equipment;
- blood test donated blood for hepatitis B and C;
- training of health personnel;
- promotion of correct and consistent use of condoms.

WHO recommends anti-HCV antibodies screening for people who may be at increased risk of infection including:

- people who inject drugs;
- people who use intranasal drugs;
- recipients of infected blood products or invasive procedures in health-care facilities with inadequate infection control practices;
- children born to mothers infected with HCV;

- people with sexual partners who are HCV-infected;
- people with HIV infection;
- prisoners or previously incarcerated persons; and
- people who have had tattoos or piercings.

For people infected with HCV, WHO recommends:

- education and counselling on options for care and treatment;
- immunization with the hepatitis A and B vaccines to prevent coinfection;
- early and appropriate medical management including antiviral therapy if appropriate;
- regular monitoring for early diagnosis of chronic liver disease.

Glossary

AHC Acute hepatitis C
ALT Alanine aminotransferase
AST Aspartate aminotransferase
CHC Chronic hepatitis C
DAAs Direct-acting antivirals
DBS Dry blood spot
EGDS Esophagogastroduodenoscopy
EIA Enzyme immunoassays
ER Endoplasmic reticulum
HCC Hepatocellular carcinoma
HCV Hepatitis C virus
IR Insulin resistance
LVP Lipoviroparticles
MC Mixed cryoglobulinemia
MTP Microsomal triglyceride transfer protein
NS Non-structural proteins
NSBB Non-selective beta blockers
POC Point of care
SBP Spontaneous bacterial peritonitis
SVR Sustained virological response
T2DM Type 2 diabetes mellitus
TIPS Transjugular intrahepatic portosystemic shunt
VLDL Very low-density lipoprotein
WHO World Health Organization

Self Study

Questions

1. What is the basis of HCV persistence?
2. What is the relevance of HCV genotypes?
3. Is the detection of anti-HCV antibodies sufficient to diagnose HCV infection and start antiviral therapy with DAAs?
4. What is the role of anti-HCV IgM and the anti-HCV avidity test for the diagnosis of AHC?

Answers

1. HCV persists in the host because of its high genetic variability, allowing the escape of the virus from the immune system. Genetic variability is a consequence of the high rate of spontaneous mutations that accumulate within the HCV genome due of the lack of proofreading activity of the HCV RNA polymerase. By continuously modifying its antigens, HCV escapes immune response. Furthermore, the inclusion of HCV in lipoviroparticles also alters antiviral response. Finally, HCV is able to spread directly from infected to non-infected hepatocytes without passing through the extracellular compartment.
2. HCV genotypes are associated with peculiar epidemiological, pathophysiological and therapeutic characteristics. Genotype 1 is more common in older patients, often has nosocomial transmission and is associated with progression to cirrhosis and HCC. Genotype 2 is prevalent in Africa and East Asia and among younger subjects, it is often transmitted by transfusion and associated with cryoglobulinemia. Genotype 3 is closely associated with the use of illicit drugs in industrialized countries and causes a severe form of hepatic steatosis associated with hypocholesterolemia. Not all DAAs are effective against all HCV genotypes and treatment outcomes may differ according to the actual genotype.
3. The detection of anti-HCV antibodies is not sufficient to diagnose an active HCV infection, but it is necessary to highlight the presence of serum HCV-RNA, therefore, treatment with DAA should only be initiated in HCV RNA positive patients.
4. IgM antibodies are not diagnostic for AHC because they can be detected both during the acute and chronic stages of the disease. Instead, the avidity test of antibodies to the anti-HCV IgG and IgM anti-HCV antibodies makes a correct diagnosis of AHC in 90% of cases.

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