

Management of hepatitis C

Alfredo Alberti^{1,2,*}, Luisa Benvegnù^{1,2}

¹Department of Clinical and Experimental Medicine, University of Padova, Via Giustiniani 2, 35100 Padova, Italy

²Venetian Institute of Molecular Medicine (VIMM), Padova, Italy

1. Introduction

Hepatitis C is a major cause of liver-related morbidity and mortality worldwide and represents a major public health problem. Its epidemiology has been changing during the last decade and great progress has been made in the development of new diagnostic tests and treatment strategies thanks to the combined efforts of the academic and industry-sponsored research.

However, there are still a number of issues that have not yet been completely solved, particularly in relation to the full understanding of the pathogenesis of liver disease and mechanisms leading to chronicity and to severe end stage. Therapy has improved in recent years and eradication of hepatitis C virus (HCV) by treatment is a reality in many chronically infected patients, but the issue of a substantial number of non-responders is still unsolved. This review addresses the main recent developments in the field of basic and clinical research on HCV, with particular reference to the clinical management of acute and chronic hepatitis C.

2. Virology and pathogenesis

Hepatitis C is caused by a small RNA virus that is included in the flaviviridae family and has been recently classified as the sole member of the genus hepacivirus [1]. HCV has a 9.6 single-stranded RNA which encodes a single polyprotein of about 3000 amino acids [2]. This HCV polyprotein is cleaved into a number of structural and non-structural proteins including: 2 envelope glycoproteins (E1 and E2), the nucleopeptide protein (core-C) and several non-structural (from NS2 to NS5) proteins.

Recent studies have classified some of the functions of these viral proteins. NS3 has helicase and protease activities while NS5 contains the RNA dependent RNA polymerase

activity. All these enzymatic activities are essential for HCV replication and are currently considered as targets for the development of new antiviral compounds [3].

Some of the viral proteins have been recently implicated also in the pathogenesis of the liver disease and in the development of resistance to interferon therapy. The HCV core proteins, which exist as full-length and truncated forms, have been shown to regulate apoptosis of infected cells [4] and might be therefore directly implicated in the pathogenesis of liver disease, of cell proliferation and liver cancer development. The core proteins, as well as NS5A, have also been reported to interfere with intracellular metabolism of lipids and of lipoproteins with a direct effect on the development of steatosis [5], which is a characteristic features of hepatitis C, particularly in patients infected with HCV-3 [6]. Last but not least, NS5A may contain a sequence domain able to regulate the cellular response to interferon. This region, which has been termed as interferon sensitivity determining region (ISDR) [7] encodes for a NS5A protein sequence that can bind and inhibit protein kinase R (PKR), a protein whose activity is of fundamental importance in the development of an intracellular antiviral state in response to interferon [8].

Despite these advances in the understanding of HCV pathogenesis, our knowledge on HCV replication and viral proteins function has been limited by the lack of suitable cell culture systems for expression and propagation of HCV and of simple small animal models of HCV infection.

Analysis and modeling of HCV kinetics during the early phase of interferon (IFN) therapy seem to indicate that the rate of virus production is quite high in patients with hepatitis C, in the range of 10^{10} – 10^{12} virions per day, with a very rapid viral turnover and a predicted half-life of 2–3 h [9]. Recently, the development of subgenomic replicons of HCV has provided a new research tool for hepatitis C [10,11]. These in vitro systems can support efficient HCV-RNA replication and synthesis of all viral proteins, although unfortunately complete virus particles production has not been yet achieved. Currently, the replicon system is actively used for testing the antiviral effect of new antiviral drugs as

* Corresponding author. Tel.: +39-049-821-2294; fax: +39-049-821-1826.

E-mail address: alfredo.alberti@unipd.it (A. Alberti).

Table 1
Assays for quantification of HCV RNA in serum^a

Assay	Manufacturer	Technique	Dynamic range of quantification
Amplicor HCV Monitor v2.0	Roche Molecular Systems, Pleasanton, CA	PCR	600– < 5 000 000 IU/ml
Cobas Amplicor HCV Monitor v2.0	Roche Molecular Systems, Pleasanton, CA	PCR	600– < 5 000 000 IU/ml
Versant HCV RNA 2.0 Quantitative Assay	Bayer Corp., Tarrytown, NJ	'Branched DNA' assay	200 000–120 000 000 genome equivalents/mla
Versant HCV RNA 3.0 Quantitative Assay	Bayer Corp., Tarrytown, NJ	'Branched DNA' assay	615–7 700 000 IU/ml
LCx HCV RNA Quantitative Assay	Abbott Diagnostics, Chicago, IL	PCR	25–2 630 000 IU/ml
SuperQuant	National Genetics Institute, Los Angeles, CA	PCR	30–1 470 000 IU/ml

^a Adapted from Pawlowsky [19].

all viral enzymes that are the major targets for antiviral therapy (NS2-3 and NS3 proteinases, NS3 helicase, NS5B RNA-dependent RNA polymerase) are encoded by the replicon RNAs. On the other hand, in the absence of complete HCV particles production, these systems have limited impact in the understanding of HCV replication and pathogenesis.

Studies conducted during natural infection in humans indicate that chronicity of hepatitis C is related to rapid production of virus, and a lack of vigorous T-cell immune response to HCV with emergence of HCV variants which are prone to escape immune control [12].

The pathogenesis of liver damage is most likely due to a combination of direct cytopathic effects of viral proteins and of immune mediated mechanisms including cytolytic and non-cytolytic reactions mediated by CTLs (cytotoxic T lymphocytes) and inflammatory cytokines [13].

Recent data indicate that oxidative stress is an important pathogenetic factor in HCV related liver damage [14]. Hepatic steatosis is also a characteristic features of hepatitis C and contributes to the progression of liver disease and fibrosis development [15].

3. Changing epidemiology and risk factors

According to most recent World Health Organization estimates, around 170–200 million individuals have chronic HCV infection worldwide. There are significant geographical variations, and significant demographical variations within the same geographic region, in the HCV prevalence.

In Europe, and particularly in the Mediterranean countries, the prevalence of HCV infection increases in parallel with age while in the United States it is most common in persons 30–49 years of age [16]. The incidence of new infections with HCV is decreasing in all Western countries while it is still high in the underdeveloped world, mainly as consequence of the use of unscreened blood transfusions and unsafe parenteral exposure. The major change in the

risk factors of HCV transmission that has occurred over time is reflected in the dramatic reduction of blood transfusion related cases and in the increasing proportion of cases due to injecting drug use. The relative importance of other exposures has changed little over time. These consist of unprotected sex with multiple partners, occupational and perinatal exposures, nosocomial and iatrogenic infections, unsafe tattooing, piercing and acupuncture [17].

According to published data and with quite significant variations in different parts of the world, no recognized source of infection can be identified in around 10–30% of infected patients.

4. Virological testing for HCV

The repertoire of virological markers that are currently used for the clinical management of patients with hepatitis C includes: antibody to HCV, detected by ELISA and immunoblot assays, serum HCV-RNA, which can be measured by highly sensitive qualitative and quantitative assays and the HCV genotype. More recently, a new marker of HCV, the HCV core antigen, has been evaluated for patients management [18].

Most of research interest for clinical application has been focused in recent years on the development and clinical validation of sensitive and specific molecular methods to measure HCV-RNA in serum. This has been mainly because of the great interest in the use of these methods for monitoring antiviral therapy. Several methods to quantify HCV-RNA levels in serum are available [19]. This has posed the problem of comparability among different assays that do not use the same units to represent the amount of HCV-RNA. To overcome such a problem, the World Health Organization has established an International standard for universal standardization of HCV-RNA quantification units [20]. The assays that are currently available for quantification of HCV-RNA in serum and their dynamic range are described in Table 1. The main use of these assays is in

the early monitoring of the virological response to antiviral therapy and in the prediction of treatment outcome. Response to α -interferon therapy and to interferon plus ribavirin combination therapy, including regimen with pegylated interferons (PEG-IFNs), is characterized by a biphasic decrease in HCV-RNA levels with an initial rapid decline occurring during the first 24–72 h, followed by a second, more gradual decline that lasts for several weeks and may lead to HCV-RNA negativity or be followed by a third, even slower, phase of HCV-RNA decline [20].

The early kinetics of these virological phases have been shown to correlate with the long term response to therapy. For this reason, the assessment of the early virological response is used to predict outcome and to identify non-responders in whom therapy should be withdrawn in order to avoid, as much as possible, the inconvenience and side-effects of therapy. The first phase kinetics shows little correlation with long-term outcomes in most published studies [21], and cannot be recommended to be used for clinical decisions, although some reports would suggest that failure to achieve at least a 1 log₁₀ drop in HCV-RNA levels during the first 24 h after initiation of therapy might correlate with virological non-response [22]. This parameter may be very much influenced by the type of regimen used. The second phase decline of HCV-RNA levels is certainly much more useful in monitoring the early virological response to therapy. The second phase decline of HCV-RNA has been classified as flat, slow or rapid. In studies where patients were treated with standard interferons or PEG-IFNs, 67% of rapid responders showed a sustained virological response compared to 27% of slow responders and 0% of flat responders [23]. Precise definition of these early virological response patterns require frequent sampling and highly standardized and reproducible HCV-RNA quantitation and is therefore not practical for routine clinical use.

Analysis of the viral response at 12 weeks in patients treated with PEG-IFNs and ribavirin combination therapy has recently led to recommending a more practical stopping rule in non-responders [24]. According to this rule, that has been proposed for monitoring PEG-IFN-ribavirin combination therapy in cases infected with HCV-1, patients should be tested by a quantitative HCV-RNA test at 12 weeks of treatment. In patients with negative HCV-RNA, therapy should be continued for 48 weeks, with a high chance of achieving a sustained virological response (SVR). Patients with a positive qualitative HCV-RNA assay at week 12 should be retested with a quantitative HCV-RNA assay and therapy should be continued only in those showing at least a 2 log₁₀ unit drop compared to baseline HCV-RNA. Therapy should be stopped in those without such a drop, as they have an extremely low chance of achieving a SVR by full course treatment. Patients showing > 2 log HCV-RNA drop should be retested by qualitative PCR at 24 weeks and continue for 48 weeks only if negative (Fig. 1).

Complete analysis of different strategies to define the early virological response during therapy and their predic-

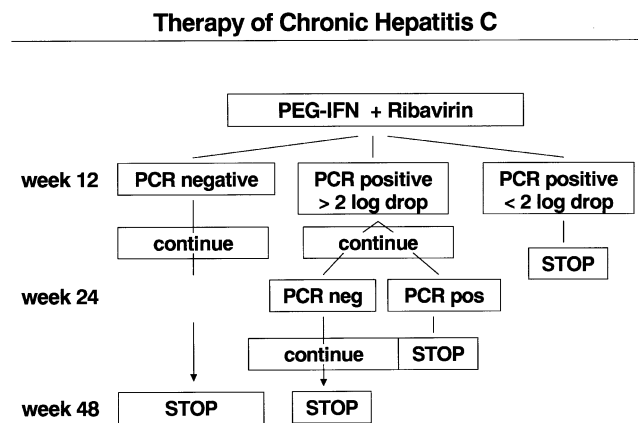


Fig. 1. Stopping rule for the treatment of chronic hepatitis C (HCV-1) with PEG-IFN plus ribavirin combination therapy.

tive value have been recently reviewed in detail by Davis et al. [24].

Many variables may influence the early virological response (EVR) and include the stage of hepatic fibrosis, gender, age, Africa-American race, and host immune status. For this reason, further studies are needed to assess the validity in other clinical settings of the stopping rule recently proposed on the basis of the results obtained with α 2b and α 2a PEG-IFNs used in combination with ribavirin in naive patients with chronic hepatitis C.

Qualitative HCV-RNA assays are the standard methods to define end of therapy response and SVR. There is general consensus that a negative HCV-RNA qualitative test result 24 weeks after stopping therapy is the most reliable marker of long-term SVR, often reflecting definitive eradication of HCV [25].

Qualitative HCV-RNA testing is also used before stopping treatment to define end of therapy virological response. However, a negative PCR assay does *not* ensure virological cure as relapse of hepatitis C may occur after therapy withdrawal in around 20–25% of patients who have achieved end of therapy virological response while treated with PEG-IFN and ribavirin. Recently a more sensitive target amplification technique (TMA) has been developed which is able to detect as little as 5–10 IU/ml of HCV-RNA. This assay is currently evaluated to detect minimal residual viremia at the end of therapy as a predictor of relapse after therapy [26,27] and as a possible tool to identify patients who may need prolongation of treatment. Further studies are needed in this field to fully understand the clinical usefulness of the TMA HCV-RNA assay during antiviral therapy.

Another virological assay currently under intense clinical investigation is the HCV core antigen EIA assay that can detect total HCV core antigen in serum. The HCV core antigen levels are expressed in pg/ml and closely correlate with HCV-RNA levels. One picogram of HCV core antigen is equivalent to approximately 8000 IU of HCV-RNA. Although the HCV core antigen assay can be used as a surrogate marker of HCV replication to monitor the early

virological response during antiviral therapy, its clinical use is limited by low sensitivity as the current version of the assay does *not* detect HCV core antigen below a HCV-RNA level of 20 000 IU/ml [28].

5. Acute hepatitis C

Due to the reduction in the incidence of new HCV infection, acute hepatitis C has become relatively uncommon in recent years. Furthermore the acute phase is often mild or completely asymptomatic, and is rarely recognized outside prospective surveillance after exposure to known risk factors. There are still no specific diagnostic tests to identify acute infection with HCV and to distinguish it from an acute exacerbation of chronic hepatitis C. For all these reasons acute hepatitis C is rarely recognized and largely underdiagnosed.

Acute hepatitis with jaundice is seen in no more than 20–25% of cases, and severe liver function impairment or failure are extremely rare events in the absence of hepatotoxic co-factors. A more severe course of acute hepatitis C can be seen in patients with excess alcohol intake, or co-infection with HBV or HIV.

An issue that has been source of great controversies during the last decade is that of the rate of chronicity after primary infection with HCV. Studies conducted in the early 1990s, mainly in patients with post-transfusion hepatitis C, indicated that most patients did not clear HCV but became chronic carriers of the virus with chronicity rates above 85–90%.

More recently, many new studies have clearly indicated that the risk of chronicity might be quite lower in other patients categories and that there are a number of cofactors and variables which affect such risk. Studies conducted in family contacts of HCV carriers and in other categories with repeated exposure to HCV have demonstrated that many of such individuals develop cellular immunity to HCV in the absence of overt infection and without anti-HCV seroconversion [29]. Such observations suggest that clearance of HCV might occur in exposed individuals much more frequently than what was thought in the past.

Although the rate of chronic outcome with virus persistence is certainly high with HCV in all patients categories, it can vary from as low as 40–50% to as high as 90–100% depending on patient age and sex (younger and female patients having a lower rate of chronicity), the source of infection and size of inoculum (the highest risk for chronicity being associated with a large e.v. inoculum like with post-transfusion hepatitis), a number of co-factors which include HBV infection, alcohol abuse and the immune status of the host. Chronicity rates are extremely high in patients with agammaglobulinemia [30]. Race is also important and higher rates of chronicity have been found in black in comparison to Caucasians and Hispanic whites in the United States [31]. A similar difference is seen also in the

response to antiviral therapy, HCV eradication being much more rarely achieved in black patients. These findings suggest that genetic factors might be important in determining the outcome of acute hepatitis C. Indeed, persistence as well as clearance of HCV after acute infection have been associated with specific immunogenetic markers.

Other studies have clearly shown that age affects the evolution to chronicity of acute hepatitis C. In the NHANES study from the United States, hepatitis C became chronic in 30% of infected subjects below the age of 20 years and 76% of those older than 20 years [32].

The clinical course and ALT profile during acute phase have also been shown to be associated with an increased risk to chronicity. Patients with symptoms and jaundice develop chronic infection more rarely than those who remain asymptomatic [33]. Furthermore, the higher the ALT peak during acute disease, the lower the probability of virus persistence. A monophasic pattern of ALT profile has also been shown to predict recovery while polyphasic ALT are often followed by chronic evolution. It should be underlined, however, that serum ALT levels may be extremely variable in acute hepatitis C and that ALT normalization after acute phase is not a reliable marker of recovery as there are patients who remain viremic despite complete and persistent normalization of ALT [34].

Some of the ALT and HCV-RNA profiles seen with acute hepatitis C are described in Fig. 2. Considering that approximately half of the patients with acute hepatitis C recover spontaneously while the other half develop chronic infection, parameters able to predict the outcome would be extremely useful in the clinical management of these cases. Unfortunately, this has not been yet adequately evaluated and it is clear that a single HCV-RNA negative sample or normal ALT during the late phase of acute hepatitis C do not prove resolution of infection and prolonged follow-up with repeated testing for at least 12 months after diagnosis is necessary to prove that the infection has resolved.

Recent studies conducted in patients with community acquired hepatitis C unrelated to blood transfusion seem to indicate that most patients who eventually recover do so within 3–4 months from clinical onset [35]. Therefore, it seems reasonable to follow patients for such a period before deciding whether or not antiviral therapy should be initiated in an attempt to prevent chronicity.

6. Should acute hepatitis C be treated?

The high propensity of acute hepatitis C to become chronic provides a strong rationale for antiviral therapy.

Published studies on the treatment of acute hepatitis C with interferon (α or β) monotherapy would indicate that therapy significantly reduces (at least by 30–40%) evolution to chronic hepatitis [36]. Unfortunately, most of these studies have been small in size, uncontrolled, and highly heterogeneous as to patient features, dose and duration of

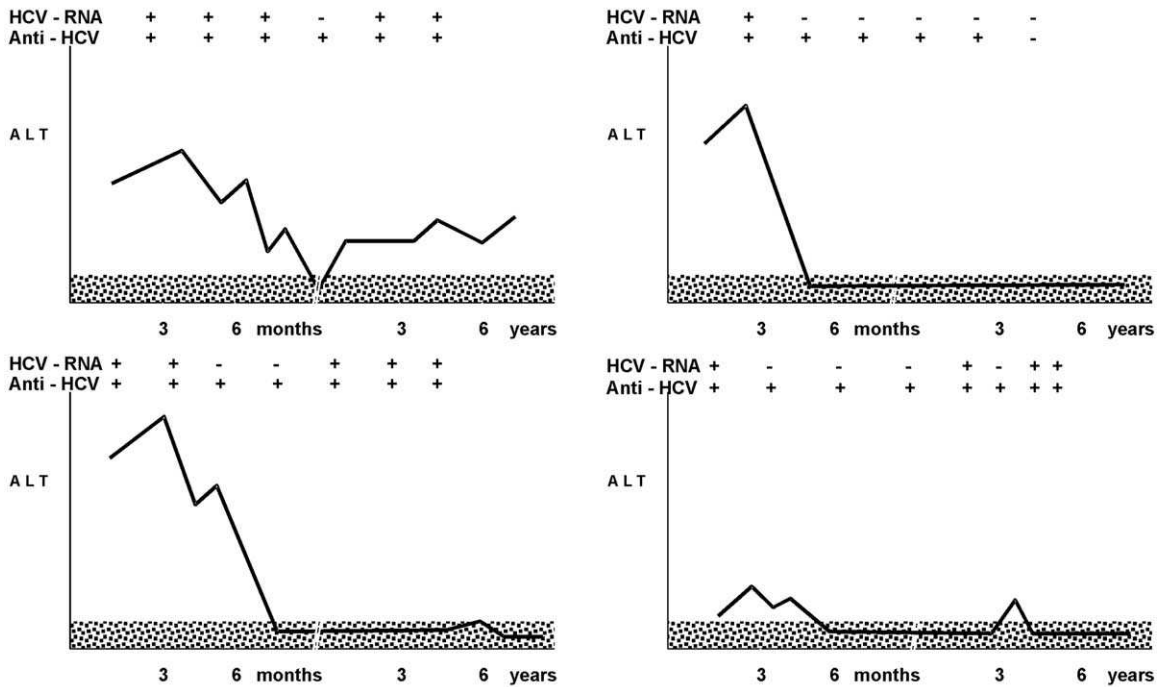


Fig. 2. Biochemical and virological outcomes of acute hepatitis C.

treatment, follow-up evaluation and criteria used to define efficacy. For these reasons, although antiviral therapy might indeed be beneficial in acute hepatitis C, it is not yet clear which patients should be treated, when therapy should be started and what regimen is optimal.

Pooling together the results of 17 published studies on the treatment of acute hepatitis C with interferon, we have recently calculated a SVR rate of 62% (range in individual studies: 37–100%) in 369 treated patients to be compared with a rate of 12% (range: 0–20%) in 201 untreated patients [36]. The highest efficacy rates were observed when daily doses of 5–10 MU of interferon α were used for 8–12 weeks, possibly followed with 5 MU given thrice weekly for a total treatment period of 24–52 weeks. At least 3

studies would indicate that delaying therapy by 2–4 months after onset to avoid unnecessary treatment of patients who recover spontaneously may not compromise efficacy in preventing chronicity [37–39].

On the basis of these observations, the most rational approach in the treatment of acute hepatitis C might be to leave patients untreated for 3–4 months after onset and then start therapy on those who remain at this point positive in serum for HCV-RNA with elevated ALT.

On the basis of available data, daily standard IFN monotherapy, or PEG-IFN monotherapy might be used for 4–6 months but further studies of adequate size and designs are urgently needed in this field to better define the optimal guidelines and regimens for the treatment of acute hepatitis C.

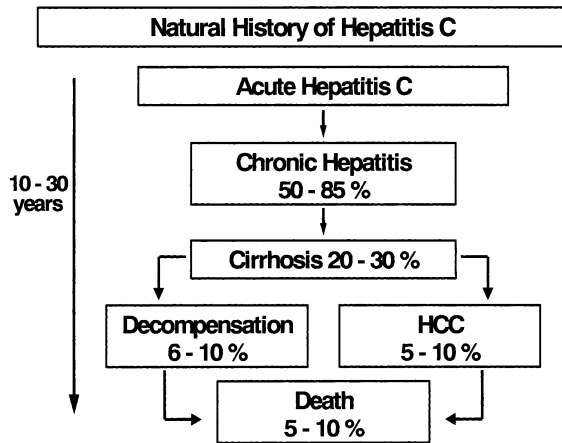


Fig. 3. General view on the natural history of hepatitis C.

7. Chronic hepatitis C: natural history and co-factors

There have been many controversies around the issue of the natural history of hepatitis C. The disease has been described as either inexorably progressive towards cirrhosis and its complications, albeit over a quite long period of time, or as a benign and non-progressive chronic infection in the vast majority of HCV carriers. Recently, it has been recognized that these discrepancies reflect the great heterogeneity of hepatitis C as to its severity and outcome and to the many cofactors that can influence its course and progression [40]. A general view of the natural history of hepatitis C is given in Fig. 3.

Retrospective studies conducted in patients with hepatitis

Table 2
Factors and variables that have been shown to influence the course of chronic hepatitis C^a

Age at infection
Gender
Race
HIV/HBV
Alcohol
Smoking
Hemochromatosis
NASH/Obesity
Schistosomiasis
Genetics
ALT profile

^a HIV, human immunodeficiency virus; HBV, hepatitis B virus; NASH, Nonalcoholic steatohepatitis; ALT, alanine aminotransferase

C observed for 10–30 years after infection indicate that 17–55% (mean 42%) developed cirrhosis, 1–23% developed HCC and 4–15% died of liver related causes. These figures are quite reduced in most prospective studies where over a follow-up period of 8–16 years after exposure 7–16% of the patients developed cirrhosis (mean 11%), 0.7–1.3% developed HCC and 1.3–3.7% died of liver related causes [41].

In a series of retrospective-prospective studies lasting 9–45 years, cirrhosis developed in 0.3–15% of the cases, HCC in 0–1.9% and liver death occurred in 0–2.8%. These studies have provided clear evidence that a number of host and environmental variables influence the course and outcome of chronic hepatitis C and explain the great heterogeneity of this disease. These differences are very well described by the quite different outcomes and rates of progression to cirrhosis seen when distinct cohort of patients were followed-up for a similar period of time (20–25 years) after infection.

In adult patients, mainly males, infected at the age of 45–65 years with a large inoculum through blood transfusion or polytransfusion in the preserologic era, 15–27% developed cirrhosis [42–44] compared to 4% with community-acquired hepatitis C [45], 1% of young drug-addicts [46], 0.4–2% of young women contaminated by anti-D Ig preparations [47] and 0.3% of children with hepatitis C [48]. These findings indicate that size and source of infection, age and gender are important variables affecting the course and outcome of chronic hepatitis C. Many other factors with an effect on the natural history of chronic hepatitis C have been identified in recent years and are summarized in Table 2.

Interestingly, virus-related variables have very little influence on the course of hepatitis C. Although HCV-1b was in the past suggested to associate with a more severe and progressive course of chronic hepatitis C, it has not been confirmed as an independent variable. At variance with what is typically seen with HIV, serum levels of HCV-RNA do not associate with the severity of hepatitis C. For these reasons, HCV genotyping and quantitative HCV-RNA measurement do not provide relevant information for clin-

ical management of untreated patients with hepatitis C. On the other hand, many host and environment factors exist that affect the course of hepatitis C.

7.1. Host related factors

Age at infection has been shown in almost all published studies to have an important impact on progression of chronic hepatitis C, that is more rapid in the elderly. Poynard et al. [49] found that only 2% of patients infected before the age of 20 years developed cirrhosis over a period of 20 years, compared to 6% of those infected between 31 and 40 years, 37% of those infected between 41 and 50 years and 63% of those who contracted HCV over the age of 50 years. In our own study [50] evaluating liver fibrosis progression in initially mild chronic hepatitis C, increasing age was associated with an increasing rate of fibrosis progression over a mean follow-up period of around 8 years (Fig. 4).

Modeling of the natural history of hepatitis C indicates that fibrosis progression is not linear over time, being slower in the younger ages of life and accelerating significantly after the age of 45–50 years, independently of other cofactors and variables.

Male gender and race have also been shown to affect disease progression in hepatitis C.

In at least three distinct studies, the percentage of patients who developed cirrhosis was lower in African-American patients (2.2% to 22%) compared to Caucasian cases (7.2% to 30%) [51–53].

Some studies have suggested that genetic factors might also play a role based on HLA class II antigen expression but this is quite controversial and has not been confirmed in all reports. In one study, B54, DRB0405 and DQB10401 were associated with disease progression while DRB11302,

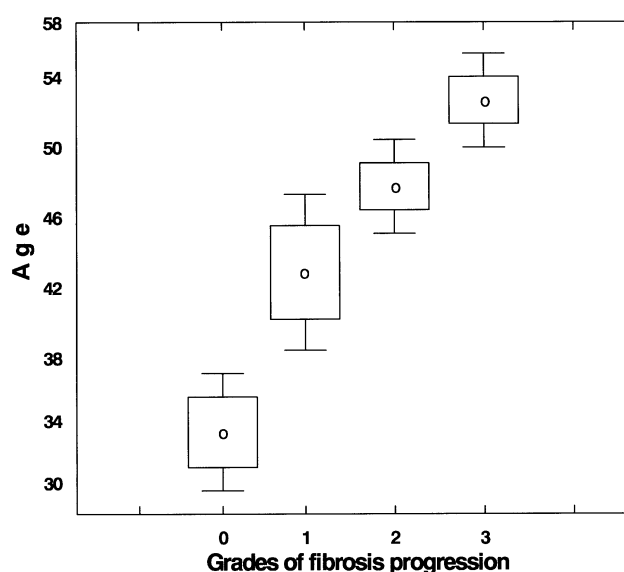


Fig. 4. Correlation between patient age and fibrosis progression serum in 106 patients with initially mild chronic hepatitis C [50].

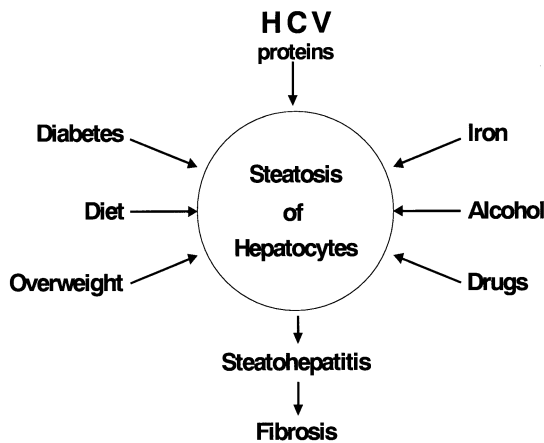


Fig. 5. Multifactorial mechanisms leading to hepatitis steatosis in hepatitis C.

DRB11101 and DQB10604 were more frequently found in HCV carriers with minimal disease and normal ALT levels [54]. Other studies have linked polymorphisms in the genes of transforming growth factors β [55], and angiotensin II [56] to a more progressive type of chronic hepatitis C.

7.2. Metabolic abnormalities and disease progression

Recently, there have been a number of reports indicating that several metabolic abnormalities and comorbidities may cause significant worsening of the clinical course of chronic hepatitis C contributing to higher rates of cirrhosis development. These conditions include increased hepatic iron stores, liver steatosis, increased body mass index and type II diabetes. Liver steatosis seems to play a central role in the pathogenesis of liver disease in hepatitis C and may be caused by many different pathways, including viral and metabolic factors (Fig. 5).

The observation that patients with chronic hepatitis C often have increased deposits of iron in the liver and that this condition is associated with more advanced liver damage and poor response to antiviral therapy, promoted a number of studies aimed at assessing the role of mutations in the hemochromatosis gene in hepatitis C. The results so far obtained have been controversial and not conclusive. Although in one study [57] patients with hepatitis C and C282Y heterozygotic mutation of the HFE gene had more advanced liver fibrosis and higher frequency of cirrhosis, other reports have not been able to confirm a significant association between mutations of the hemochromatosis gene and severity of hepatitis C [58,59]. Nevertheless, from a practical point of view it seems reasonable to evaluate iron metabolism and overload in patients with chronic hepatitis C, particularly in the presence of more advanced fibrosis, and to remove the excess by phlebotomy, whatever the cause of iron overload might be, as this approach has been shown to reduce ALT levels and disease progression and to improve the response to antiviral therapy.

During the past 2 years, great interest has been generated by a number of clinical studies indicating that liver steatosis might play a most relevant role in hepatitis C. Liver steatosis is a frequent finding in the biopsy of patients with HCV infection and in some of them it might be extensive and severe. In our own study [60], based on the analysis of more than 400 patients with chronic hepatitis C, steatosis was detected in 65% and was of grade 3 (severe) in 23%. Patients infected with HCV-3 had higher rates and grades of steatosis compared to cases with HCV-1 or HCV-2, in agreement with published data [61,62].

The mechanisms leading to lipid accumulation in the hepatocytes of HCV infected patients appear multifactorial. In a subgroup of patients liver steatosis is associated with risk factors of non-alcoholic fatty liver (NAFL) or steatohepatitis (NASH) such as obesity, or type II diabetes, while in other cases such factors are absent and liver steatosis might be directly related to the virus itself (viral steatosis). A 'metabolic' type of steatosis is more frequently seen in patients with HCV-1 or HCV-2 while the 'viral' type is typically seen with HCV-3 and might be related to the direct effect of viral proteins (core protein and NS5A) which interfere with the intracellular uptake and transport of triglycerides and with the assembling and secretion of lipoproteins.

Whatever the cause, liver steatosis may contribute to progression of fibrosis in patients with HCV. Indeed, obesity with a body mass index (BMI) > 30 kg/m², liver steatosis of moderate/severe grade and type II diabetes have all been associated with more advanced liver disease [63–65].

These data clearly indicate the need for assessing liver steatosis in patients with hepatitis C and considering dietetic and/or therapeutic strategies able to prevent or reduce it, to be used side by side with more specific antiviral therapies.

7.3. Environmental and external factors

Co-infection with HBV or HIV has been shown to accelerate the course of chronic hepatitis C and facilitate progression to cirrhosis and hepatocellular carcinoma. Therefore, patients with HCV should be tested for HBV and HIV markers in the presence of risk factors, as such co-infections may modify the clinical management and therapeutic strategies. Little is known about how to treat patients with HBV/HCV co-infection. As the two viruses may often interfere in their replicative activity [66], testing for HBV-DNA and for HCV-RNA in serum should be performed in all cases to identify the dominating virus and treat the patients accordingly using an 'HCV' protocol in HCV-RNA positive/HBV-DNA negative cases and an 'HBV' protocol in HCV-RNA negative/HBV-DNA positive patients. In patients with evidence of active replication of both viruses, our strategy has been to give first interferon plus ribavirin to eradicate HCV, followed by HBV treatment in the case of a persistent HBV-DNA positive test. Clinical trials of adequate size are clearly needed to better define how and whom to treat with HBV/HCV co-infection.

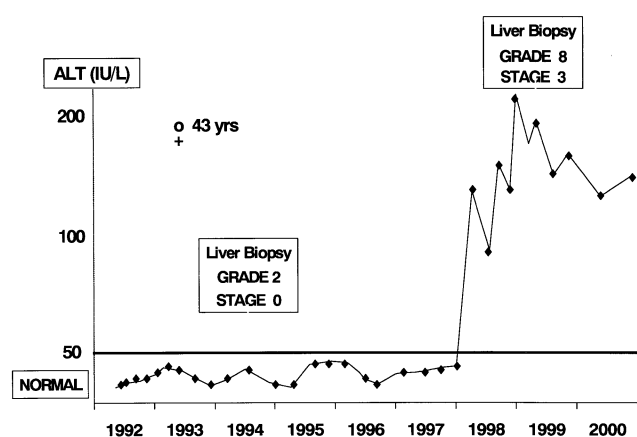


Fig. 6. Reactivation of hepatitis C after 6 years of persistently normal ALT.

Co-infection with HCV/HIV is common [67]. Approximately 25% of subjects infected with HIV are also infected with HCV. Since the introduction of highly active antiretroviral therapies (HAART) the overall mortality for AIDS and related opportunistic co-infections has declined sharply and liver disease due to HCV has emerged as an important cause of death in HCV/HIV co-infected patients. HIV co-infection has been shown to increase the risk of HCV chronicity and accelerate progression of liver disease.

On the other hand, HCV and the related liver disease often complicate the clinical management of HIV patients by increasing the grade of immune impairment and the liver and extrahepatic toxicity of antiretroviral drugs. Therefore, management of HCV infection in HIV positive patients has become of great importance. A liver biopsy is usually needed to evaluate stage of chronic hepatitis C. Ideally, HCV antiviral therapy should be started before HAART, when the HIV infection stage allows to do so. HIV co-infected patients do appear to respond to interferon monotherapy as non-co-infected patients when the pretreatment CD4 cell count is above 500/mm³. Ongoing studies with IFN plus ribavirin and PEG-IFNs will provide in the near future more evidence based guidelines for the management and treatment of HIV/HCV co-infected individuals.

7.4. Alcohol and HCV

Although alcohol abuse has been clearly shown to increase the severity of hepatitis C, with more rapid progression to cirrhosis and hepatocellular carcinoma, these effects relate mainly to patients with quite high daily alcohol intake. Much less is known on whether a light or moderate amount of alcohol might also exert a negative influence on HCV. These issues have been extensively discussed recently by Peters and Terrault [68]. These authors have clearly underlined the need of future studies addressing the issue of the effect of minimal to moderate alcohol intake (one or two drinks per day or less than 20 g daily) on the course of hepatitis C and response to antiviral therapy.

8. Liver disease and clinical management in HCV carriers with normal ALT

Approximately 30% of patients with chronic HCV infection have normal ALT levels at diagnosis, independently of age and gender [69] and many of them do maintain normal enzyme levels during prolonged follow-up, although reactivation of liver disease with ALT flares may be seen in a significant number of cases (Fig. 6). Some of these HCV carriers with normal ALT have significant fibrosis or cirrhosis on liver biopsy. Presence of significant liver disease or the risk of future ALT reactivation cannot be predicted by virological or biochemical testing.

Table 3 summarizes the incidence of significant liver disease, that is of active inflammation and/or advanced fibrosis, seen in the liver biopsy of asymptomatic HCV carriers with normal (or nearly normal) ALT in published series. Overall, around 20% have significant histologic findings. On the other hand, recent prospective studies indicate that another 15–25% will eventually show reactivation of biochemical activity during a 3 month to 10 years follow-up [70,71].

In front of these data and preliminary evidence that interferon (or PEG-IFN) plus ribavirin combination therapy is effective and safe in this category of patients as in those with elevated ALT, HCV carriers are becoming more and more motivated to receive antiviral therapy, independently of the severity of their liver disease and of the short term prognosis. According to the conclusions and recommendations of the most recent Consensus Conference on the Management of hepatitis C, organized by the National Institutes of Health in June 2002, HCV carriers who present with normal ALT should not be excluded ‘a priori’ from the possibility

Table 3
A compendium of published studies assessing liver disease stage and activity in HCV carriers with normal ALT

Authors	No. of cases ALT normal	No. (%) with significant fibrosis on biopsy
Alberti [93]	16	6 (37%)
Conry-Cantilena [94]	72	5 (7%)
Esteban [95]	105	14 (13%)
Gholson [96]	50	20 (40%)
Kolho [97]	20	5 (25%)
Naito [98]	22	0 (0%)
Okanone [99]	36	2 (5.5%)
Prati [100]	41	0 (0%)
Puoti [101]	46	12 (26%)
Serfaty [102]	85	51 (60%)
Shakil [103]	51	21 (41%)
Shindo [104]	19	0 (0%)
Silini [105]	48	0 (0%)
Yuki [106]	21	0 (0%)
Total	632	136 (22%)

of being treated [72]. The decision to treat or not to treat should be taken on the basis of a set of variables, including liver histology, patient age and motivation, the HCV genotype and load, as well as co-morbidities and contraindications. When therapy is decided, these patients should be treated as those with elevated ALT, waiting for more data on the optimal treatment schedule and monitoring that will derive from ongoing clinical trials.

9. Natural history and management of initially mild chronic hepatitis C

A subgroup of patients with chronic hepatitis C have little or no fibrosis in the initial liver biopsy and are referred as cases of 'mild' chronic hepatitis C. Such patients can have elevated or, more frequently, normal or nearly normal ALT. The ALT levels, however, have only limited correlation with histologic findings and liver biopsy remains the only tool to precisely define the stage (fibrosis) and grade (inflammatory activity) of hepatitis C.

The natural history of initially mild chronic hepatitis C is only partially understood. At short term follow-up, most of these patients show very little evidence of progression of liver fibrosis and development of cirrhosis is extremely rare. On the basis of such findings, it has been repeatedly suggested that patients with histologically mild chronic hepatitis C should not be treated with antivirals but rather be followed up prospectively with sequential liver biopsies to identify those with disease progression and reserve treatment to such subgroup. These strategies might not be always the most rational and cost-effective in the light of several considerations:

1. The significant improvement in the efficacy of antiviral therapies for chronic hepatitis C recently obtained with IFN plus ribavirin and with PEG-IFN plus ribavirin combination therapy.
2. The evidence that these treatments are more effective and better tolerated in patients with mild and well compensated liver disease.
3. The evidence that most patients with initially mild chronic hepatitis C show progression of liver fibrosis when followed up for 3–10 years. In three large series of patients with initially mild chronic hepatitis C who were left untreated and were followed-up prospectively and evaluated for disease progression with sequential liver biopsies [73–75], fibrosis progression was seen in around 30% of the cases when the second liver biopsy was taken within 3–4 years after the initial one, and in most of the cases fibrosis worsening was only of 1 grade. On the other hand, in our own series of 106 patients followed for longer with sequential liver biopsies taken 7–11 years apart, fibrosis did progress in 60%, and 36% reached an advanced stage of fibrosis and 16% did develop cirrhosis [75].

On the basis of these considerations, immediate therapy might be preferred to a wait-and-see strategy, at least in a subgroup of patients with mild chronic hepatitis C considering patient motivation and pros and cons of treatment.

The recent NIH Consensus Conference on the Management of Hepatitis C, although had recommended a wait and see strategy for mild chronic hepatitis C, has also suggested that young patients, highly motivated and infected with 'easy to treat' HCV (HCV-2 or HCV-3), should be considered for therapy independently of liver biopsy findings, in the absence of contraindications [72].

10. New standards of therapy for chronic hepatitis C

Therapy of chronic hepatitis C has greatly improved in recent years. Rates of sustained virological response have increased significantly in the late 1990s with the addition of ribavirin to α -interferon and have further improved more recently with the use of PEG-IFNs, again in combination with ribavirin. The recent NIH Consensus Conference of the Management of Hepatitis C has concluded that on the basis of available data the highest response rates to antiviral therapy for the treatment of chronic hepatitis C have been achieved using the combination of PEG-IFNs and ribavirin, at least for patients infected with HCV-1 and such regimen has been therefore proposed as the new standard of therapy for chronic hepatitis C.

The pegylated forms of IFN have been developed with the aim of prolonging the half-life of recombinant interferon α 2b and α 2a from a few hours, as seen with the original non-pegylated forms used for many years either alone or in combination with ribavirin, to several days as seen with PEG- α 2b IFN and PEG- α 2a IFN. The prolonged half-life of these new formulations allows once-a-week administration and results in improved pharmacokinetics and pharmacodynamics of the drug, with more profound and efficient suppression of virus activity compared to standard IFNs administered thrice weekly.

Two forms of PEG-IFNs have been developed and evaluated as monotherapy or in combination with ribavirin in large multicenter studies involving several hundreds of patients. These two PEG-IFNs differ as to physicochemical structure and properties: PEG- α 2b (PEG-INTRON, Schering-Plough Corp., Kenilworth, NJ) is IFN- α 2b bound to a linear molecule of polyethylene glycol of 12 kDa size while PEG- α 2a (Pegasys, Hoffmann-La Roche, Nutley, NJ) is IFN- α 2a linked to a much larger and branched 40 kDa molecule of PEG. These differences result in different pharmacokinetics and pharmacodynamic properties, PEG- α 2a having a longer half-life and a smaller body distribution space compared to PEG- α 2b. This latter drug is cleared mainly by the kidney while the former is removed mainly by the liver.

Despite these differences, efficacy of the two drugs was quite similar in registration trials, particularly when used in

combination with ribavirin. In these studies PEG-IFNs were compared with standard IFN, being used alone as monotherapy or in combination with ribavirin.

The results of the major clinical trials of PEG-IFN based therapy in chronic hepatitis C are summarized in Table 4. Monotherapy studies [76–78] clearly indicated that both forms of PEG-IFNs were significantly superior to standard IFNs in relation to ETVR and SVR rates. Accordingly, the SVR with PEG-IFN- α 2a was 39% compared to 19% with standard interferon in non-cirrhotic patients [76] and 30% compared to 8% in patients with advanced liver disease (bridging fibrosis or cirrhosis) [77] when a dose of 180 μ g of PEG-IFN was used once-a-week, with highly statistically significant difference. In a similar study, different dosage of PEG-IFN- α 2b, adjusted by body weight and given once a week were compared with 3 MU thrice weekly of the old formulation of IFN- α 2b. Treatment was for 1 year with SVR of only 12% with standard IFN, 18% with 0.5 μ g/kg

per week of PEG-IFN, 25% with 1.0 μ g/kg per week of PEG-IFN and 23% with 1.5 μ g/kg per week [78].

In these monotherapy studies SVR with PEG-IFNs correlated with the same variables that were shown in the past to predict SVR to standard IFN monotherapy, i.e. age less than 40 years, absence of cirrhosis or bridging fibrosis, lower body surface area and, most significant, the HCV genotype and pretreatment viral load.

The next step was obviously that of combining PEG-IFNs with ribavirin and to compare such combination with standard IFN plus ribavirin treatments. Three large pivotal trials of PEG-IFN plus ribavirin combination therapy have been conducted [79–81] and the results are summarized in Table 4.

The main findings were:

1. On intent-to-treat analysis, rates of sustained virological response were almost identical with PEG-IFN- α 2b (54%) or PEG-IFN- α 2a (56%) combined with ribavirin

Table 4
Randomized controlled trials of PEG-IFNs therapies^a

	Regimen	No. of patients	ETVR (95% CI)	SVR (95% CI)	
PEG-IFN monotherapy studies					
Zeuzem et al., 2000 [76]	IFN- α 2a, 6 mU tiw for 12 wks, then 3 mU for 36 wks	264	28% (22–33)	19% (14–24)	
	PEG-IFN- α 2a, 180 μ g weekly for 48 wks	267	69% (63–75)	39% (33–45)	
Heathcote et al., 2000 [77]	IFN- α 2a, 3 mU tiw for 48 wks	88	14% (8–23)	7% (4–16)	
	PEG-IFN- α 2a, 90 μ g weekly for 48 wks	96	42% (32–52)	15% (9–23)	
	PEG-IFN- α 2a, 180 μ g weekly for 48 wks	87	44% (34–54)	30% (21–40)	
Lindsay et al., 2001 [78]	IFN- α 2b, 3 mU tiw for 48 wks	303	24% (20–29)	12% (9–16)	
	PEG-IFN- α 2b, 0.5 μ g/kg weekly for 48 wks	315	33% (28–39)	18% (14–23)	
	PEG-IFN- α 2b, 1.0 μ g/kg weekly for 48 wks	297	41% (35–46)	25% (20–30)	
	PEG-IFN- α 2b, 1.5 μ g/kg weekly for 48 wks	304	49% (43–55)	23% (19–28)	
PEG-IFN plus ribavirin combination studies					
Manns et al., 2001 [79]	IFN- α 2b, 3 mU tiw and ribavirin (1000–1200 mg) for 48 wks	505	54% (49–58)	47% (42–51)	
	PEG-IFN- α 2b, 1.5 \rightarrow 0.5 μ g/kg weekly, and ribavirin (1000–1200 mg) for 48 wks	514	56% (52–60)	47% (43–52)	
	PEG-IFN- α 2b, 1.5 μ g/kg weekly, and ribavirin (800 mg) for 48 wks	511	65% (61–69)	54% (49–58)	
Fried et al., 2002 [80]	IFN- α 2b, 3 mU tiw and ribavirin (1000–1200 mg) for 48 wks	444	52% (47–57)	44% (40–49)	
	IFN- α 2a, 180 μ g weekly and placebo for 48 wks	224	59% (53–66)	29% (24–36)	
	IFN- α 2a, 180 μ g weekly and ribavirin (1000–1200 mg) for 48 wks	453	69% (65–73)	56% (52–61)	
				HCV-1	Non HCV-1
Hadziyannis et al., 2002 [81]	IFN- α 2a, 180 μ g weekly and ribavirin (800 mg) for 24 wks	207	Not available	29% (21–38)	78% (70–85)
	IFN- α 2a, 180 μ g weekly and ribavirin (800 mg) for 48 wks	361	Not available	40% (34–46)	73% (64–80)
	IFN- α 2a, 180 μ g weekly and ribavirin (1000–1200 mg) for 24 wks	280	Not available	41% (32–50)	78% (71–84)
	IFN- α 2a, 180 μ g weekly and ribavirin (1000–1200 mg) for 48 wks	436	Not available	51% (45–57)	77% (70–83)

^a ETVR, end therapy virological response; SVR, sustained virological response; CI, confidence interval; IFN, interferon; PEG-IFN, pegylated interferon; tiw, thrice weekly; wks, weeks.

- and were significantly higher than those observed with standard IFN plus ribavirin combination therapy.
2. Pre-treatment variables that correlated with SVR were HCV genotype 2 and 3, low HCV-RNA levels, body weight, age and degree of liver fibrosis.
 3. In the studies conducted with PEG-IFN- α 2b post hoc analysis showed that SVR was dependent on the ribavirin dose expressed as mg/kg body weight with a threshold value of 10.6 mg/kg below which rates of SVR become unsatisfactory.
 4. The benefit of using PEG-IFN instead of standard IFN in combination with ribavirin were not uniform in different patients subgroups: PEG-IFN- α 2b improved significantly SVR in patients with HCV-1 and low viral load but not in those with HCV-2 or HCV-3 or HCV-1 and high viral load, while more homogeneous, although limited (+6–10%) increments in SVR were seen with PEG-IFN- α 2a compared to standard IFN.
 5. In one study using PEG-IFN α 2a in combination with ribavirin for 24 or 48 weeks [81], it was clear that patients with HCV-1 improved significantly their SVR when treated for longer, independently of pretreatment viral load while no such difference was seen for patients with HCV-2 or HCV-3 again independently of pretreatment HCV-RNA levels. Furthermore, a fixed dose of 800 mg of ribavirin was sufficient to maximize SVR rates in patients with HCV-2/3 while patients with HCV-1 responded better to higher dosages (1000–1200 mg/daily) of ribavirin.
 6. With both PEG-IFNs it was clear that adherence to the intended dosage of IFN and ribavirin and duration of therapy is important to maximize SVR rates, particularly for HCV-1 patients with high viral load and during the early phase of therapy, although this information was derived from retrospective analysis of the data and might be biased.
 7. With both types of PEG-IFN, tolerability and side effects were similar to those seen with standard IFN, with the exception of bone marrow toxicity and particularly neutropenia that was more frequently seen with PEG-IFNs, particularly when used in combination with ribavirin.

On the basis of the results obtained in these trials, both forms of PEG-IFNs have been approved in the United States and Europe for the treatment of chronic hepatitis C. The current recommendation is to use combination therapy with PEG-IFNs and ribavirin as new standard of treatment for all cases of chronic hepatitis C, except in situations where there are contraindications to ribavirin. The recommended dose of PEG-IFN- α 2a is 180 μ g/week, independently of body weight, while that of PEG-IFN- α 2b is weight adjusted and fixed at 1.5 μ g/kg per week with combination therapy and 1.0 μ g/kg per week with monotherapy. The dose of ribavirin and the duration of therapy should be decided according to the HCV genotype. Patients

with ‘easy-to-treat’ HCV (HCV-2 or HCV-3) should be given a fixed dose of 800 mg daily of ribavirin and should be treated for 24 weeks while those with ‘difficult-to-treat’ HCV (HCV-1 and, possibly, HCV-4) should be given a full dose of ribavirin (1000–1200 mg daily based on body weight less than or greater than 75 kg) and treated for 48 weeks.

Many issues remain unresolved on how to treat chronic hepatitis C with these new strategies. The optimal dose of PEG-IFN- α 2b for combination therapy should be further explored as the dose of PEG-IFN- α 2a and - α 2b to be used in patients with HCV-2 or HCV-3 who might be often over-treated with currently used regimens.

Another issue to be addressed is whether PEG-IFNs should be used on body weight adjusted doses or fixed dosage. Furthermore, currently recommended schedules and monitoring of response have not been sufficiently explored and validated in several patients subgroups such as those with compensated cirrhosis, impairment of the immune system or associated comorbidities. More data on the effectiveness and tolerability of these new treatments are needed from community based studies before generability of the results obtained in registration trials can be made.

One major unsolved issue is what to do with those patients who do not respond to therapy.

11. Retreatment of hepatitis C

Patients who have failed a first course of antiviral therapy differ in their chance of benefiting from retreatment according to:

1. The type and duration of first treatment
2. The type of response achieved during first treatment

Patients who have been relapsers after a first cycle of IFN monotherapy can be retreated with IFN plus ribavirin combination therapy (with standard IFN or PEG-IFNs) with a reasonably high chance of achieving SVR [82]. Retreatments should be conducted with the same schedules proposed for naive patients. Patients who have been non-responders to a first course of IFN monotherapy are more difficult to retreat but 12–20% can achieve SVR when given IFN plus ribavirin for 48 weeks [83]. Retreatments with PEG-IFN and ribavirin can increase SVR rates to 35–40% according to preliminary data of ongoing studies [84]. The variables that correlate with SVR after retreatment are identical to those found to predict SVR in naive patients and include HCV genotype, rate, stage of fibrosis in the liver. In a recent multicenter study, 212 non-responders to either IFN alone or IFN plus ribavirin combination were retreated with PEG-IFN- α 2a and ribavirin for 1 year [85]. SVR was seen in 34% of IFN non-responders and in only 11% of IFN/ribavirin non-responders. Overall, only 15% of patients with HCV-1 compared to 60% of those with HCV-2 or -3

developed SVR with retreatment. On the basis of these findings, PEG-IFN plus ribavirin combination therapy given at full dosages for 48 weeks appears as a rationale retreatment approach for IFN non-responders while current strategies are clearly unsatisfactory for IFN/ribavirin non-responders. In these patients, and particularly in those with a resistant type of HCV-1 infection, new strategies are urgently needed.

New approaches under evaluation include small molecule inhibitors of HCV enzymes most relevant for virus replicative activities (protease, helicase and polymerase) new immune modulators, new ribavirin analogs deprived of hematological toxicity, ribozymes and antisense molecules. In this field, interesting preliminary results have been recently reported on the antiviral efficacy of an oral HCV serine protease inhibitor in patients with HCV-1, including non-responders to IFN and/or IFN plus ribavirin standard therapies [86].

This novel compound, BILN2061, was administered to 31 patients with HCV-1 and minimal liver fibrosis at different doses (from 25 to 500 $\mu\text{g}/\text{daily}$) for 2 days, in comparison with a placebo group. Viral load decreased by 1 \log_{10} unit or more in almost all but two treated patients with no changes in the placebo group. After the end of therapy, HCV-RNA returned to pretreatment levels in all cases. Interestingly, the antiviral effect was similar in naive patients and in previous IFN or IFN plus ribavirin non-responders. This is the first report on the clinical use of a protease inhibitor for HCV and particularly in the scenario of IFN/ribavirin non-responders. However, the results presented, although of great interest, were very preliminary and further data on long-term efficacy and tolerability of this and of similar compounds are clearly needed and will derive from ongoing clinical trials.

12. Long-term ‘suggestive’ therapy in non-responders

While waiting for new treatment strategies able to eradicate HCV in patients who do not have a virological response with currently available therapy, it has been suggested that such patients could benefit from long-term administration of interferon, with the aim of reducing disease activity, fibrosis progression and abnormal hepatocyte proliferation, thus reducing or delaying progression to cirrhosis and development of hepatocellular carcinoma [87].

This is still a quite controversial issue and although several retrospective cohort studies would suggest that virological non-responders might have marginal benefit from long-term administration of interferon, this has not yet been proven by properly designed prospective studies, that are currently ongoing in the United States and Europe with PEG-IFNs. Until the results of such studies are available, the use of IFN therapy as suppressive treatment for hepatitis C should be considered ‘experimental’ and cannot be recommended in the clinical practice.

13. HCV vaccines: lights and shadows

The development of an effective HCV vaccine remains an unsolved challenge. During the last decade, many efforts have been dedicated to the design of either prophylactic or therapeutic HCV vaccines, but still with little success. These issues have been extensively reviewed most recently in the *Journal of Hepatology* [88].

Development of HCV vaccines has been and is still difficult for several reasons:

1. The immunological correlates of recovery from HCV infection are only partially understood, due to the limited accessibility to acute phase convalescent samples.
2. HCV is highly heterogeneous, and often escapes the host immune response by rapid mutation. Neutralizing antibodies are produced in acute infection but they are often isolate- or strain-specific and do not provide protection against subsequent exposure [89].
3. There is a lack of suitable cell culture systems and of small animal models to establish HCV infection and replication for evaluating crucial virus neutralizing pathways.

Many different approaches have been and are currently tested using structural (E1–E2-core) and non-structural HCV proteins as candidates for different strategies of immunization [88,90,91]. New adjuvants are also evaluated to improve immunogenicity and to favor the generation of a strong cellular response [92]. While there is currently great skepticism on the possibility of obtaining soon an effective prophylactic HCV vaccine able to provide wide protection against the infection, greater hopes are posed on the development of a therapeutic vaccine able to potentiate cellular immune response in chronically infected patients and to terminate the infection, used either alone or in combination with other antiviral therapies.

Declaration

The authors state that they do have a relationship with the manufacturers of the drugs mentioned in the paper but have not received funding to carry out this study.

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