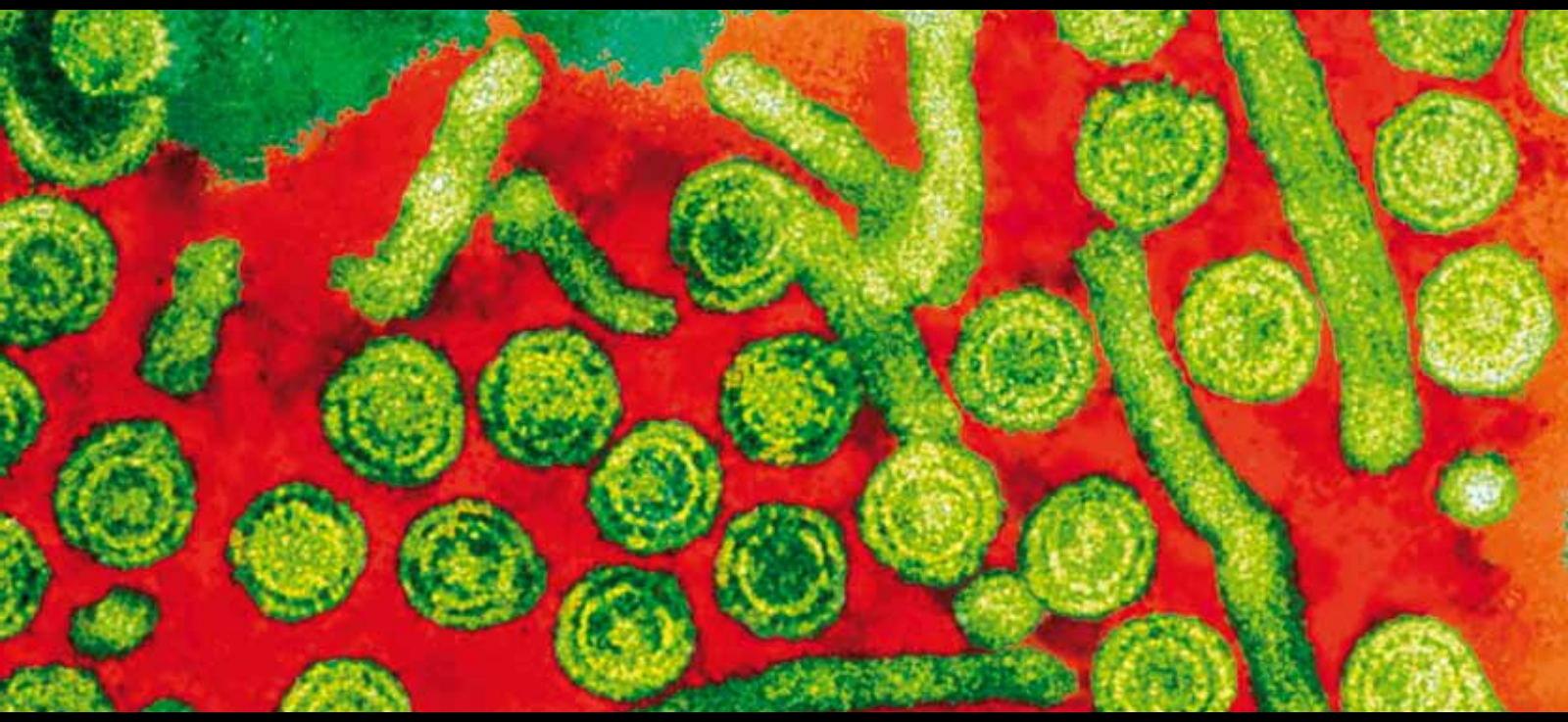
Hepatitis Research and Treatment

Pegylated Interferon and Ribavirin Treatment for Hepatitis C Virus Infection

Guest Editors: TateHiro Kagawa, Emmet B. Keeffe, and Yu Ming-Lung



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Editorial Board

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Hindawi Publishing Corporation
Hepatitis Research and Treatment
Volume 2010, Article ID 275274, 2 pages
doi:10.1155/2010/275274

Editorial

Pegylated Interferon and Ribavirin Treatment for Hepatitis C Virus Infection

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Received 25 November 2010; Accepted 25 November 2010

We are pleased to serve as editors of this special issue. We were gratified by the excellent response to the call for papers and the high quality of the eleven manuscripts that were ultimately accepted for this special issue.

This issue begins, as is appropriate, with a historical perspective by R. M. Friedman and S. Contente from the Uniformed Services University of the Health Sciences on treatment of chronic hepatitis C with interferon, followed by a paper by C.-H. Chen and M.-L. Yu from Kaohsiung Medical University tracing the evolution of interferon-based therapy, including the addition of ribavirin and pegylation of interferon. Both of these papers provide an interesting capsule of where we began with the initial recognition of the antiviral activity of interferon in 1957, followed by the evolution interferon-based therapy of chronic hepatitis C with the addition of ribavirin in the mid-1990s and the demonstration of the superiority of pegylated interferon (peginterferon) plus ribavirin in the early 2000s. The sustained virologic response (SVR) rate increased from 8%–9% with interferon monotherapy to 30% in genotype 1 patients with the addition of ribavirin, and then to 40%–50% with the transition to peginterferon plus ribavirin. As noted in several papers in this special issue, we are on the threshold of increasing the SVR rate in 2011 up to 65%–75% with the addition of a protease inhibitor to the standard of care with peginterferon plus ribavirin. Thus, advances in the treatment of chronic hepatitis C continue to march forward, with SVR rates increasing from less than 10% with interferon monotherapy to close to 75% in the very near future with triple therapy using a protease inhibitor. What is remarkable is that this progress has all taken place within the past 20 years. In the next paper, T. Kagawa and E. Keeffe review the recent literature evaluating the long-term outcomes of antiviral therapy in patients with chronic hepatitis C that convincingly demonstrate the impact of interferon-based treatment of chronic hepatitis C. The published data shows slowed disease progression in patients who achieve an SVR, including improved inflammation and fibrosis scores on follow-up biopsy, reduced incidence of hepatocellular carcinoma, and prolonged life expectancy with reduced liver-related deaths. Thus, the progress in treatment over the past 20 years is paying dividends to our patients.

The prediction of response to interferon-based therapy is an important issue for patients considering embarking on a course of therapy, and providers of care continue to improve their ability to predict success in individual patients by taking into account baseline host (age, race, gender, histology (stage of fibrosis and presence or absence of steatosis), body weight, insulin resistance, and *IL28B* genotype) and viral factors (genotype and HCV RNA level). A number of papers in this special issue address predictors of viral response. N. Izumi et al. provide a thorough and scholarly review of the various factors that predict an SVR and their relative importance. R. Cubillas et al. from Southwestern Medical Center in Dallas elegantly demonstrate that tumor necrosis factor receptor 1 is upregulated in dendritic cells in patients with chronic hepatitis C who respond to therapy. Although not clinically available, R. Kubota et al. demonstrate that

erythrocyte ribavirin levels can predict which patients are more likely to achieve an early virologic response after 12 weeks of treatment.

A number of miscellaneous papers in this special issue address the treatment of chronic hepatitis C in a number of special populations. M. Numata et al. from several Japanese medical centers treated 122 patients with genotype 1 infection and demonstrated by multivariate analysis that adherence to peginterferon and ribavirin was the only predictor of SVR. The rate of SVR fell sharply when exposure to peginterferon was less than 80% and also decreased in a stepwise fashion when ribavirin exposure was 60%–80% and less than 60% compared with greater than 80%. D. F. Meyer et al. from several New York medical centers report that daily high-dose consensus interferon (24 μg) plus weight-based ribavirin was not successful in genotype 1 nonresponders to prior therapy and was associated with substantial side effects. Y. Y. Hwang and R. H. S. Liang from Queen Mary Hospital in Hong Kong review antiviral therapy in hematologic patients and point out that the published literature demonstrates that HCV RNA levels increase during chemotherapy and immunosuppression and that there is a risk of rebound immunity against hepatitis C with liver injury after discontinuation of immunosuppression. They recommend that close monitoring during chemotherapy is appropriate and that antiviral therapy with peginterferon and ribavirin should be deferred until complete of chemotherapy and recovery of immunity. H. Weclawiak et al. focus on the management of chronic hepatitis C during hemodialysis and reinforce the recommendation to treat during dialysis, as there is a high rate of SVR that eliminates recurrence of HCV infection after kidney transplantation. They also reinforce the standard recommendation not to treat chronic hepatitis C after kidney transplantation because of the high risk of acute allograft rejection. Finally, Y. Sugawara et al. from the University of Tokyo provide a thorough review of the controversies and challenges in the management of recurrent chronic hepatitis C after liver transplantation. Although there is no general consensus on who, when and how to treat, the overall SVR rate is 25%–45% with standard peginterferon plus ribavirin, in spite of a high prevalence of intolerability. The newer antiviral therapies in 2011 may bring greater success in the management of patients with posttransplant recurrent HCV infection.

The editors are confident that the readers will enjoy reading this special issue and the diverse topics that are expertly reviewed.

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Hindawi Publishing Corporation
Hepatitis Research and Treatment
Volume 2010, Article ID 323926, 4 pages
doi:10.1155/2010/323926

Review Article

Treatment of Hepatitis C Infections with Interferon: A Historical Perspective

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Received 13 April 2010; Revised 2 July 2010; Accepted 30 July 2010

Academic Editor: Ming-Lung Yu

Interferons were first described in 1957, but it was not until 34 years after their discovery that sufficient quantities of it were available for treatment of hepatitis C virus (HCV) infections, Clinicians now have an excellent understanding of the basis for the effectiveness of interferon alpha (IFN-α) in the therapy of this disease. Treatment with IFN-α is more efficient when it complemented by the antiviral ribavirin and the IFN-α is conjugated with polyethylene glycol to form peginterferon. In the near future treatment of HCV with IFN-α may involve new anti-HCV agents that are currently under development.

The antiviral activity of interferon (IFN), first described in 1957, was in a chick cell and inactivated influenza virus system [1]. The inactivated virus induced a protein that had a broad spectrum of antiviral activity, which immediately attracted wide interest, so that there was expectation that interferons (IFNs) rapidly would develop clinically as agents to treat a range of viral infections. In addition to their antiviral activity, IFNs were later discovered to be important regulators of both cellular growth and the immune response. A number of problems arose, however, that delayed their clinical use for the treatment of virus infections for many years. The first of these was that the IFNs, with some exceptions, are species-specific in their biological activity [2], so that only human or primate interferons were found to be active in humans. This meant that the single source of interferons for human use in the 1960s and 70s was primate cells, and the supply of such cells was quite limited. Another problem was that IFNs could only be assayed by means of their ability to inhibit virus replication in a tissue culture system [1]. In addition, IFNs were found to possess then unprecedented biological activity, and it became evident that existing stocks of IFNs with very significant antiviral activity actually were quite impure and so contained very little IFN. Because of the lack of even moderately clean IFN, it was impossible to accept any biological activity of an IFN preparation, other than antiviral activity, as being due to its IFN content, although subsequently IFNs were shown to have many biological functions. Despite such problems, and because of the promise IFNs held as a possible treatment for viral diseases, there were early clinical trials of the antiviral activity of what IFN preparations were then available. These studies tested the ability of an IFN produced by simian cells to inhibit the development of vaccinia virus lesions in human skin or respiratory infections following exposure of volunteers to common cold viruses [3, 4]. The results were unimpressive, almost certainly because of the small quantities of impure IFN used, so that for many years studies on IFNs were limited to experiments in tissue culture and to attempts to produce and purify sufficient quantities of IFN from human cells to carry out significant clinical studies. To further complicate matters, it was discovered that there were actually several forms of human IFN, IFNs-α, -β, and -γ. There are seven subtypes of human IFN-α, but only single genes coding for IFNs-β and -γ. Subsequently, additional forms were discovered, but only IFNs -α, -β, and -γ are presently used clinically.

Interest in IFNs was reignited in the mid-1970s when sufficient quantities of fairly clean human IFN-α, obtained by Cantell's group in Finland from the white blood cell buffy coats of donated blood, became available [5] for clinical

experiments. Many of these had promising, if not highly significant, results in studies on the prevention of common colds [6] and the treatment of several herpes virus infections, such as herpes keratoconjunctivitis and the varicella-zoster infections, shingles and chickenpox [7, 8]. The discovery that in tissue culture experiments mouse IFN-β inhibited chronic infections with mouse leukemia viruses [9] prompted additional studies employing interferon as therapy for human chronic hepatitis B virus (HBV) infections. These had very promising results [10].

A 1974 report that Cantell's IFN-α was an effective treatment for cancer, although later shown to be flawed, nevertheless had profound effects on interferon research, both positive and negative [11]. That IFNs might be potential anticancer drugs led to widespread, unwarranted, and later disappointed expectations of their being a general cure for cancer; on the positive side, however, interest in finding better sources for a potential wonder drug led directly to the cloning of genes for human IFN-α [12], and later for IFNs-β and -γ [13, 14]. This in turn led to the production of quantities of pure IFNs sufficient to carry out a large number of clinical trials with significant results. Such studies have partially clarified what the role of IFNs might be in the treatment of several diseases. Recombinant IFN-αs presently are widely employed with some success in the treatment of chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections and with limited effectiveness, in some forms of neoplasia such as melanomas [15]. IFN-β treatment is regularly used to limit exacerbations of multiple sclerosis [16]. IFN-γ has been approved for clinical use only in a rare congenital disorder, chronic granulomatous disease, for which it is effective in preventing recurrent bacterial infections. Current clinical trials are underway employing in the treatment of chronic HBV and HCV infections IFN-λ, which is biologically similar to IFNs-α and -β, but employs a different membrane receptor [17, 18]. Phase 1 trials for IFN-λ were successfully completed in October, 2009, and Phase 2 trials have been initiated.

By far the best understood clinical application of IFNs biologically is against chronic HCV infections, for which IFN-α has been an approved treatment since 1991, although IFN treatment for HCV was first employed in 1986 with some promise, well before the viral cause of the infection had been identified [19, 20]. HCV is a widespread infection spread by contaminated blood products or by drug injection. Although modern blood bank technology has almost eliminated the former, the latter remains a major problem. There are worldwide millions of HCV-infected patients. The progress of HCV infections is insidious, often not being clinically manifest for two or three decades after initial infection with the virus. Chronic HCV infection may cause serious hepatic malfunction eventually resulting in cirrhosis of the liver and in life-threatening esophageal varices. In addition, a significant number of patients with chronic HCV infections eventually develop hepatocellular cancers (hepatomas) and have an increased risk for developing renal cell carcinomas [21]. Chronic infections with HCV are a significant cause of death in patients with AIDS [22].

HCV is a small Flavivirus, the sole member of the hepacivirus ribovirus species, with seven genotypes, of which genotype 1, unfortunately the most common infection in North America, is relatively insensitive to IFN-α. It appears possible to predict the response of a patient to infection with a genotype 1 HCV isolate by use of structural analysis of the infecting virus [23]. The core protein of genotype 1 HCV induces cellular proliferation and transformation and so is associated with advanced hepatic cirrhosis and hepatocellular transformation [24]. The resistance of HCV to IFN resides in a nonstructural viral protein NS3/4A, a serine protease that inactivates the signal leading to interferon production, thus apparently facilitating the development of chronic infections [25]. IFNs-α and -β production is induced when a cellular protein receptor, RIG-1 (retinoic acid inducible gene), is activated by single-strand virus RNA. Activated RIG-1 in turn interacts with the adaptor mitochondrial antiviral signaling (MAVS) protein that phosphorylates IFN response factor 3 (IRF3), leading eventually to production of IFN [26]. The viral NS3/4 protease inhibits interferon production by hydrolyzing the attachment of MAVS to its site on mitochondria. HCV growth is, however, sensitive to the antiviral action of IFN although the mechanism for this inhibition is presently uncertain. It may involve two of the proteins induced by IFN treatment, a ribonuclease that destroys HCV RNA or a protein kinase that inactivates a factor required for virus protein synthesis [27]. The expression of the gene for IL-28B, which codes for IFN-λ, is a predictor of the ability of patients to clear HCV or to respond to therapy for HCV infections [28]. Patients with severe cases of HCV appear to respond to IFN therapy better than do patients with more moderate infections [29].

In order to augment the effectiveness of IFN-α employed in the treatment of HCV, two alterations in the protocol for its treatment were initiated. Ribavirin, an oral purine analogue that inhibits the growth of some RNA viruses, such as flaviviruses, either by inhibiting the HCV polymerase or by inducing lethal virus mutations among other possible mechanisms, was added to the regimen [30]; and IFN-α was conjugated to polyethylene glycol to yield peginterferon. This conjugation decreases the renal clearance of the IFN and so significantly increases its half-life from about 5 h to almost 90 h, which in turn allows a reduction in the required frequency of treatments [31, 32]. Of the two forms of peginterferon available, peginterferon alfa-2a appears to be somewhat more effective than does peginterferon alfa-2b [33, 34]. With the combined ribavirin/peginterferon treatment, more than 75% of nongenotype 1 HCV patients maintain a sustained anti-HCV response, and up to 50% of the patients infected with the genotype 1 HCV responded to this combined treatment; in those patients responding to peginterferon/ribavirin therapy, virus-induced liver damage failed to progress, with some degree of healing taking place [31]. IFN-based treatment was associated with improved survival and reduced the risk of hepatocellular cancer. Long-term followup indicated that once a particular HCV-infected patient attains a sustained response to peginterferon/ribavirin therapy, defined as undetectable levels of HCV RNA in the serum for six months, the risk for virologic

relapse is very low [35]. In one clinical study, low doses of peginterferon and ribavirin were as effective as higher dose levels [24, 36].

Current treatments for chronic HCV infections have several limitations, as they result in rates of sustained virus responses that were lower in black and Latino patients than in non-Latino whites [37, 38]. Long-term, IFN-based treatment did not halt the progression of chronic HCV infections in patients not responding to initial treatment [36]. A variable percentage of patients treated with IFN develop anti-IFN antibodies, but surprisingly, there appears to be little correlation between the presence of such antibodies and the response to IFN [39]. IFN-α is also useful in the treatment of cryoglobulinemia and focal glomerulopathy, complications of chronic HCV infections [40].

Unfortunately, the prolonged peginterferon therapy necessary to control chronic HCV or HBV infections was often associated with serious side effects such as fatigue, fever, and myalgias, symptoms of many acute virus infections, possibly because such effects are due to the induction of IFNs by the infecting agents. Usually these symptoms respond to treatment with nonsteroidal anti-inflammatory agents [27]. In some patients, treatment with IFNs has also resulted in psychiatric problems such as depression, anxiety, and excessive irritability that may require treatment with psychoactive pharmaceuticals. More severe toxicities, such as cytopenias and autoimmune disorders, also have rarely been reported in patients treated with IFNs [41].

In patients who did not respond to standard peginterferon/ribavirin therapy, substitution of the consensus interferon, alfacon-1, plus ribavirin proved effective in some cases [42]. Currently, new forms of therapy to augment treatment with ribavirin/peginterferon are under development, including inhibitors of the HCV protease, helicase, or polymerase and IFN-α conjugated to albumin [43]. Telaprevir, an inhibitor of the HCV nonstructural protease NS3/4, has proven to be effective when employed with peginterferon/ribavirin to treat patients with chronic HCV infections that are unresponsive to conventional peginterferon/ribavirin therapy [44]. Combinations of additional new agents with the currently employed therapies may provide effective treatment for a much larger percentage of HCV patients than are currently responding to anti-HCV treatment [31].

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Hindawi Publishing Corporation
Hepatitis Research and Treatment
Volume 2010, Article ID 140953, 12 pages
doi:10.1155/2010/140953

Review Article

Evolution of Interferon-Based Therapy for Chronic Hepatitis C

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Received 15 May 2010; Accepted 23 August 2010

Academic Editor: Tatehiro Kagawa

Since 1986, interferon-alfa (IFN-α) monotherapy has been administered for patients with chronic hepatitis C (CHC). However, sustained response rate is only about 8% to 9%. Subsequent introduction of ribavirin in combination with IFN-α was a major breakthrough in the treatment of CHC. Sustained virological responses (SVRs) rate is about 30% in hepatitis C virus genotype 1 (HCV-1) patients, and is about 65% in HCV-2 or -3 patients. After 2000, pegylated interferon (PegIFN) much improved the rates of SVR. Presently, PegIFN-α-ribavirin combination therapy has been current standard of care for patients infected with HCV. In patients with HCV-1, treatment for 48 weeks is optimal, but 24 weeks of treatment is sufficient in HCV-2 or -3 infected patients. Clinical factors have been identified as predictors for the efficacy of the IFN-based therapy. The baseline factor most strongly predictive of an SVR is the presence of HCV-2 or -3 infections. Rapid virological response (RVR) is the single best predictor of an SVR to PegIFN-ribavirin therapy. If patients can't achieve a RVR but achieve a complete early virological response (cEVR), treatment with current standard of care can provide more than 90% SVR rate. HCV-1 patients who do not achieve an EVR should discontinue the therapy. Recent advances of protease inhibitor may contribute the development of a novel triple combination therapy.

1. Introduction

Interferon-alfa (IFN-α) monotherapy has been found with normalization of alanine aminotransferase (ALT) levels in a few patients diagnosed as non-A, non-B hepatitis even before hepatitis C virus (HCV) was identified as the chief etiologic agent in this diagnosis [1]. In 1989, the first cases of successful treatment of documented chronic hepatitis C (CHC) with IFN-α monotherapy were reported, but relapse after the cessation of treatment was common [2, 3]. The introduction of combination therapy with IFN-α and ribavirin has markedly improved treatment response. Nevertheless, less than one-half of patients with CHC were able to experience a favorable response to the combination therapy [4–6]. Since 2000, the attachment of inert polyethylene glycol to conventional IFN-α, pegylated IFN-α (PegIFN-α), reduced degradation and clearance, prolonging the half-life of IFN and permitting less frequent, weekly dosing while maintaining higher sustained IFN levels (compared with 3 times weekly for conventional IFN). Now, PegIFN-α-ribavirin combination treatment has been recommended for all patients infected with HCV. For patients infected with HCV genotype 1 (HCV-1), the recommended treatment duration is 48 weeks, whereas for patients infected with HCV-2 or HCV-3, the recommended treatment duration is 24 weeks [7].

2. Approved Agents for Treatment of Hepatitis C

2.1. IFN-α. IFNs are natural cellular proteins with a variety of actions. There are two distinct but complementary mechanisms for the antiviral effects of IFN-α: (a) induction of a non-virus-specific antiviral state in infected cells, resulting in direct inhibition of viral replication, and (b) immunomodulatory effects that enhance the host's specific antiviral immune responses and may accelerate the death of infected cells [8]. A number of different IFNs have been used [9]. The U.S. Food and Drug Administration (FDA) has approved 3 IFN preparations for treatment of HCV: (a) 3 million units (MUs) IFN-α-2a 3 times weekly; (b) 3 MUs

of IFN-α-2b 3 times weekly; and (c) 9 μgs of IFN alfacon-1 twice weekly, or 15 μg 3 times weekly in nonresponders [10].

2.2. Peginterferon (PegIFN). PegIFN is a product of pegylation to conventional IFN (the attachment of inert polyethylene glycol (PEG) polymers to a therapeutic protein such as IFN). The larger molecular size of the compound results in a longer half-life due to reduced clearance, while retaining biological activity, and allows more convenient once-weekly dosing. Two PegIFNs [11, 12] were studied: (a) PegIFN-α-2a, a 40 kDa branched molecule with a terminal half-life of 80 hours (range: 50–140 hours) and a mean clearance of 22 mL/hr·kg administered at a fixed 180 μg per week and (b) PegIFN-α-2b, a 12 kDa linear molecule with a mean terminal half-life of 40 hours (range: 22–60 hours) and a mean clearance of 94 mL/hr · kg, administered on the basis of weight (1.5 μg/kg/week). Maximal serum concentrations ($C_{\max}$) occur between 15 and 44 hours post dose and are sustained for up to 48–72 hours. These two PegIFNs much improved the rates of SVR in comparison with their nonpegylated counterparts [11, 12].

2.3. Ribavirin. Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is an oral purine nucleoside analogue with broad activity against viral pathogens [13]. Clearance of ribavirin is markedly reduced with renal insufficiency [14]. The mechanism of action of ribavirin in CHC remains controversial. Among the suggested, but not proven, roles of ribavirin in the treatment of CHC are an immunologic modulation through switching the T-cell phenotype from type 2 to type 1; inhibition of host inosine monophosphate dehydrogenase activity; depletion of intracellular guanosine triphosphate pools; induction of mutational catastrophe; or a moderate, transient, early direct antiviral effect [15]. Ribavirin may lead to rapid and lethal mutation of virions or depletion of intracellular guanosine triphosphate, which is necessary for viral RNA synthesis [16]. Additionally, ribavirin may act synergistically with IFN by upregulating the activity of double-stranded RNA-activated protein kinase and enhances the action of interferon-alfa against hepatitis C virus [17].

The most interesting clinical observation is that ribavirin monotherapy had a minimal effect on HCV viremia, despite the fact that serum ALT levels were reduced significantly in a considerable proportion of patients with chronic HCV infection [18]. However, the combination of ribavirin and IFN provides a clinically synergistic anti-HCV effect. Hence it was proposed that ribavirin may exert its effect on the host immune response. Several studies on virus-specific T-cell reactivity in association with IFN treatment have found increased numbers of patients with CHC with demonstrable HCV-specific Th responses either during treatment or after a sustained therapeutic response. These findings raise the possibility that enhancement of HCV-specific T-cell reactivity may be one mechanism for successful antiviral treatment. HCV-specific T-cell reactivity was uncommon at baseline but increased markedly during antiviral therapy, peaking around treatment weeks 4–8 [19]. The main difference in T-cell reactivity of patients treated with IFN-ribavirin was a significant decrease of the expression of IFN-Υ, whereas INF-Υ expression was similar to that in patients receiving IFN monotherapy. The greater efficacy of ribavirin may exert an anti-inflammatory effect and may help reducing IFN-γ-driven T-cell activation and liver damage [20].

3. Assessment of Treatment Response for Hepatitis C

In earlier studies, the primary end point for HCV therapies was a biochemical response, defined as the normalization of ALT levels [2, 3]. The introduction of virologic assays to detect HCV RNA further allows the assessment of a virologic response, defined as polymerase chain reaction- (PCR-) seronegative ($\leqq$50 IU/mL, or 100 copies/mL) for HCV RNA. Histological response has been assessed in some clinical studies, but there is little indication for posttreatment biopsy in clinical practice.

Four on-treatment and three patterns of off-treatment virological responses to antiviral therapy for hepatitis C have emerged over the past decade [21–23]. They include the following:

(1) *rapid virologic response (RVR):* PCR-seronegative of HCV RNA at week 4;

(2) *early virologic response (EVR):* there are two kinds stratifications of EVR:

  (a) complete EVR (cEVR): PCR-seronegative of HCV RNA at week 12;

  (b) partial EVR (pEVR): decrease of HCV RNA by >2 log from baseline values at week 12;

(3) *end-of-treatment virologic response (ETVR):* PCR-seronegative of HCV RNA at the end of therapy;

(4) *virologic breakthrough:* HCV RNA reappearance in serum while still on treatment;

(5) *sustained virologic response (SVR):* PCR-seronegative of HCV RNA 6 months after completing therapy;

(6) *virologic Relapse:* PCR-seronegative of HCV RNA at the end of therapy, with return of circulating virus after completion of therapy;

(7) *nonresponders:* persistently seropositive for HCV RNA throughout treatment.

More than 97% of patients with SVR remain nonviremic by PCR for the subsequent 5–14 years [24, 25]. These patients are regarded as having a high probability of a durable biochemical, virologic, and histological response [26].

4. Evolution of IFN-Based Therapy for Chronic Hepatitis C

4.1. IFN-α Monotherapy. Until the 1990s, the only therapy of proven benefit for patients with CHC was IFN-α. Initially, a 6-month course of 3 weekly injections of 3 MUs of IFN-α was

approved for treatment of CHC, and a biochemical response, defined as the normalization of ALT levels, was assigned as the primary end point [2, 3]. IFN-α monotherapy suppresses serum HCV RNA to undetectable levels and normalizes the ALT level in 25% to 40% of CHC patients, usually within the first 2-3 months of treatment. However, these initial responses to IFN-α monotherapy are usually transient, and sustained response is documented in only about 8% to 9% of patients [27].

When virologic assays for detection of HCV RNA became available, the virological response rates were observed to be lower than those reported with biochemical end points. In the meta-analysis of IFN-α monotherapy [28], normalization of ALT levels at the end of treatment and 6 months after stopping treatment was seen in 47% and 23% of treated patients, respectively. ETVR and SVR, however, were observed in only 29% and 8% of treated patients, respectively. Improvement of efficacy on CHC could be achieved with higher doses and/or a longer duration of IFN-α monotherapy. A doubling of the duration of therapy to 12 months increased the frequency of SVRs to approximately 20%. The best efficacy/risk ratio was in favor of 3 MUs of IFN-α 3 times weekly for at least 12 months in treatment-naïve patients with CHC [27].

4.2. IFN-α and Ribavirin Combination Therapy. The introduction of ribavirin in combination with IFN-α was a major breakthrough in the treatment of CHC. Even though ribavirin monotherapy was shown to be ineffective [18], the rate of SVRs was 43% and 6% for the IFN-α-2a with and without ribavirin combination [4], respectively, and 36% and 18% for the IFN-α-2b with and without ribavirin combination [29]. A meta-analysis in 1995 showed that the SVR rate was significantly higher for IFN-ribavirin combination therapy than for IFN or ribavirin monotherapy (odds ratio [OR]: IFN-ribavirin versus IFN = 9.8; 95% confidence interval [CI] = l.9–50) [30].

Several landmark studies then followed and consistently demonstrated the dramatically improved responses to combination therapy, especially for HCV-2 or HCV-3 patients. In 1998, two multicenter randomized controlled trials (RCTs) (one U.S. study and one international study) totaling 1,744 previously untreated patients with compensated CHC compared 24- and 48-week drug regimens of IFN-α-2b monotherapy (3 MUs 3 times weekly) with those of IFN-α-2b and ribavirin (1.000 mg/day or 1.200 mg/day for patients weighing <75 kg or >75 kg, resp.) combination therapy followed by 24 weeks of off-therapy followup [5, 6]. The overall SVR rates for 24 and 48 weeks of therapy were 33% and 41%, respectively, for patients receiving IFN-α-2b-ribavirin, compared with SVR rates of 6% at 24 weeks and 16% at 48 weeks IFN-α-2b monotherapy. In addition to definitively showing the benefit of combination therapy over IFN alone, these studies made several other important clinical points. First, a striking reduction in hepatic inflammation was seen in sustained virological responders. Second, the likelihood of response to treatment was related to pretreatment virus level and genotype. SVRs to 48 or 24 weeks of combination therapy occurred in 29% and 17% of HCV-1 patients, respectively, and in 65% and 66% of HCV-2 or HCV-3 patients. The two studies reinforced the importance of longer duration therapy for 48 weeks in patients with HCV-1 infection. Similarly, SVRs to 48 or 24 weeks of combination therapy occurred in 38% and 27% of patients with pretreatment HCV RNA levels of greater than 2×10^6 copies/mL, respectively, but the SVR rates were no different for those with lower levels (45% and 43%, resp.). A systematic review in 2001 included data from 15 trials in which patients received IFN-α monotherapy or IFN-α-ribavirin combination therapy. In comparison with IFN-α monotherapy, combination therapy reduced the nonresponse rate (absence of SVR) by 26% in treatment-naïve patients (relative risk = 0.74; 95% CI = 0.70–0.78). Morbidity and mortality showed a nonsignificant trend during treatment in favor of combination therapy.

In 1998, the FDA approved the combination of IFN-α and ribavirin for patients with chronic HCV infection. In 1999, the EASL International Consensus Conference on Hepatitis C recommended that, for patients with CHC who have not been previously treated, (a) standard therapy should consist of IFN-α and ribavirin in combination for 24 weeks and that (b) treatment should be extended to 48 weeks in patients with both HCV-1 and HCV RNA levels greater than 2×10^6 copies/mL [31].

4.3. PegIFN-α Monotherapy. Four RCTs compared the efficacy and safety of once-weekly PegIFN-α monotherapy compared with IFN-α monotherapy three times per week for the treatment of chronic HCV infection in treatment-naïve patients [11, 12, 32, 33]. The initial studies of PegIFN-α evaluated the dose-ranging efficacy of monotherapy. The recommended dose of PegIFN-α-2a monotherapy, administered fixed at 180 μg/week for 48 weeks, achieved higher SVR rates compared with IFN-α-2a monotherapy (30% to 39% versus 8% to 19%) [12, 32, 33]; the PegIFN-α-2b monotherapy, administered according to body weight at 1.5 μg/kg/week for 48 weeks, achieved an SVR rate of 23%, compared to 12% with IFN-α-2b monotherapy [11].

Of note, Heathcote et al. [32] conducted the first substantive prospective study confined to patients with compensated cirrhosis or advanced fibrosis. Cirrhosis has been a poor predictor of responsiveness and is associated with a high risk of leucopenia and thrombocytopenia [5, 6]. This study, however, showed that PegIFN monotherapy was both well tolerated and effective in cirrhotic CHC patients, with an SVR rate of 30%.

PegIFN monotherapy has been recommended for patients with contraindications to ribavirin, such as those with renal insufficiency, hemoglobinopathies, and ischemic cardiovascular disease. Some clinical trials have been reported to date in these populations [34, 35]. For patients with contraindications to ribavirin but who have indications for antiviral therapy, PegIFN represents the best option of treatment.

4.4. PegIFN-α and Ribavirin Combination Therapy. The results of PegIFN-α monotherapy encouraged more clinical

trials to go on and anticipation that combination therapy with PegIFN-α and ribavirin would be even more effective. The earlier two large RCTs were applied with fixed durations of 48 weeks [36, 37]. In these trials, PegIFN-α-2b was dosed by weight (1.5 μg/kg was FDA approved) and coupled with 800 mg of ribavirin; PegIFN-α-2a was given at a fixed dose of 180 μg along with a weight-adjusted, higher dose of ribavirin (1.000 mg/day or 1.200 mg/day for patients weighing <75 kg or >75 kg, resp.). The overall response rate in clinical trials was 54% to 56%. These trials demonstrated that higher SVR rates could be achieved with the combination of PegIFN-α weekly plus oral ribavirin given twice daily than with the combination of IFN-α given 3 times weekly plus ribavirin or than with PegIFN-α monotherapy.

The issue of influence of ribavirin dose by body weight on the response rate was first addressed. In the PegIFN-α-2b study, a post hoc analysis demonstrated that an SVR of 61% was achieved in the subgroup whose daily dose of ribavirin exceeded 10.6 mg/kg. Logistic regression analyses observed that the response rates generally increased as ribavirin dose increased up to about 13 mg/kg/day. Actually, the optimal ribavirin dose has not been defined. Some studies highlighted the potential importance of higher doses of ribavirin [38, 39]. The first 4 weeks of weight-based ribavirin exposure (>13 mg/kg/day) have been associated with the achievement of an RVR [40]. In non-RVR patients, one post hoc analysis showed that providing and maintaining optimal dose of ribavirin within 12 weeks of treatment was pivotal for the attainment of a cEVR [41]. Patients with a cEVR in this study received a ribavirin dose of 16.3 mg/kg/day. Moreover, a higher weight-based dose of ribavirin (15.2 mg/kilogram/day) was associated with a lower relapse rate and higher SVR rate [42].

Later, the optimal treatment duration and ribavirin dose were investigated in a multicenter RCT in which all CHC patients received PegIFN-α-2a at a dose of 180 μg, while patients in the four arms received either 24 or 48 weeks of ribavirin at a dose of 800 mg or at the higher, weight-based doses of 1.000 or 1.200 mg daily [43]. In the subsequent registration trial, a high frequency of SVRs occurred in patients with HCV-2 or HCV-3, regardless of the regimen (79% to 84%), but optimal frequencies of SVRs in HCV-1 (52%) required longer duration and full-dose ribavirin, independent of the level of HCV RNA. In patients with HCV-1 with a low viral load ($<2 \times 10^6$ copies/mL, or 800.000 IU/mL), the SVR was highest in those who had received the higher ribavirin dose and who were treated for 48 weeks (61%). This regimen was also optimal for patients with HCV-1 and a high viral load (SVR rate: 46%). In contrast, in patients with HCV-2 or HCV-3, regardless of the pretreatment viral load, no differences were detected with the 4 treatment regimens. Another single-arm, open-label, historical-control study of 24 weeks of treatment with PegIFN-α-2b plus ribavirin limited to patients with HCV-2 or HCV-3 demonstrated that 24 weeks of treatment was sufficient in HCV-2- or HCV-3-infected patients, with an overall SVR rate of 81% [44]. This study supports the current recommendations that patients with HCV-1 require 48 weeks of PegIFN-α therapy with higher doses of ribavirin, while patients with HCV-2 or HCV-3 can be treated for only 24 weeks and with only 800 mg daily of ribavirin [7, 45].

So far, there are 3 RCTs to compare the rates of SVR of these two PegIFNs. One RCT showed no significant difference between the two available peginterferon-ribavirin regimens in patients infected with HCV genotype 1 [46]. Two RCTs showed that SVR rates were significantly greater in HCV patients treated with PegIFN-α-2a than patients treated with PegIFN-α-2b [12, 47, 48]. One recent meta-analysis showed that peginterferon alpfa-2a significantly increased the number of patients who achieved a sustained virological response (SVR) versus peginterferon alfa-2b (47% versus 41%; risk ratio: 1.11; 95% confidence interval: 1.04–1.19; $P = .004$ (eight trials)) [49].

4.5. Contraindication and Adverse Events of IFN-Ribavirin and Management. Contraindications and adverse events of IFN-ribavirin therapy are listed in Table 1. Physicians should look specifically for contraindications to antiviral therapy and assess both therapeutic risk and benefit. Ribavirin is contraindicated in pregnancy, necessitating strict precautions and contraception in women of childbearing age and their sexual partners and in HCV-infected men with female partners of childbearing age. Flu-like side effects of IFN can be managed with acetaminophen or nonsteroidal anti-inflammatory drugs; antidepressants and hypnotics can be used for depression and insomnia, respectively. For management of neutropenia, dose reduction suffices; the addition of granulocyte colony-stimulating factor is generally not recommended, although it may be considered in individual cases of severe neutropenia. Treatment with ribavirin should be avoided in patients with ischemic cardiovascular and cerebrovascular disease and in patients with renal insufficiency. If anemia occurs, options include ribavirin dose reduction or the addition of erythropoietin. Patients with decompensated cirrhosis are at high risk of adverse events and relatively contraindicated to IFN-ribavirin.

Patients receiving combination therapy had an increased risk for requiring medication dose reduction (RR = 2.44; 95% CI = 1.58–3.75) or discontinuation (RR = 1.28; 95% CI = 1.07–1.52) compared with those receiving IFN monotherapy [50]. The rates of IFN dose reduction and discontinuation were similar among subjects receiving PegIFN and conventional IFN [11, 12].

5. Factors Associated with Treatment Efficacy

With the great progress in the management of CHC, clinical factors have been identified as predictors for the efficacy of the IFN-based therapy. They could be divided into two major categories: baseline and on-treatment predictors (Table 2).

5.1. Baseline Predictors of Response to IFN-Based Therapy

5.1.1. Virologic Factors. The pretreatment variable most strongly predictive of an SVR is the presence of HCV-2 or HCV-3 infection [51], whether with conventional IFNs or PegIFNs, alone or in combination with ribavirin [5, 6, 36, 37]. On the basis of variations in the nucleotide sequence

Table 1: Contraindications and adverse effects of hepatitis C therapy.

Contraindications	
Absolute contraindications	Major, uncontrolled depressive illness; autoimmune hepatitis or other condition known to be exacerbated by interferon and ribavirin; untreated hyperthyroidism; pregnant or unwilling/unable to comply with adequate contraception; severe concurrent disease such as severe hypertension, heart failure, significant coronary artery disease, poorly controlled diabetes, obstructive pulmonary disease; under 3 years of age; known hypersensitivity to drugs used to treat HCV
Relative contraindications	Decompensated liver disease; solid organ transplantation (except liver); coexisting medical conditions: severe anemia (hemoglobin level < 100 g/L), neutropenia (neutrophil count < 0.75×10^9/L), thrombocytopenia (platelet count < 40×10^9/L), hemoglobinopathy, uncontrolled heart disease (angina, congestive heart failure, significant arrhythmias), cerebrovascular disease, advanced renal failure (creatinine clearance < 50 mL/min)
Adverse effects	
Interferon or peginterferon	Flu-like symptoms (fever, fatigue, myalgia and headaches); mild bone marrow suppression (especially, leucopenia and thrombocytopenia); gastrointestinal manifestation (anorexia, nausea, vomiting and diarrhea); emotional effects (depression, irritability, difficulty concentrating, memory disturbance and insomnia); dermatological manifestation (skin irritation, rash and alopecia); autoimmune disorders (especially thyroid dysfunction); weight loss; tinnitus and hearing loss; retinopathy (usually not clinically significant); hyperglycemia; seizures; renal function impairment; pneumonitis.
Ribavirin	Hemolytic anemia (dose dependent); cough and dyspnea; rash and pruritis; nausea; sinus disorders; teratogenicity.

Table 2: Factors associated with response to interferon-based therapy for hepatitis C.

Baseline
Virological factors
Hepatitis C virus genotype
Hepatitis C viral loads
Quasispecies
Host factors
Bridging fibrosis/cirrhosis
Gender
Age
Ethnicity
Insulin resistance
Obesity
Hepatic steatosis
Host genetics: genetic variation in IL28B
Coinfection with HIV
Nonresponse to previous interferon-based therapy
On-treatment
Rapid virological response (RVR) at week 4
Early virological response (EVR) at week 12
Complete EVR (cEVR) versus Partial EVR (pEVR)
Medical adherence

of HCV, six genotypes (numbered 1–6) and more than 50 subtypes (identified by lowercase letters, e.g., 1a and 1b) have been identified [52]. Why HCV-1 is harder to treat than other HCV genotypes is not yet fully understood. Several studies demonstrated that there exists a genotype-specific difference of viral kinetics [23, 53]. The turnover of hepatocytes infected with HCV-1 is slower than that of hepatocytes infected with other HCV genotypes after initiation of IFN-based therapy [53, 54], implying that HCV-1 might be more resistant to antiviral therapy. Under the current recommendation [7], SVR rates were 42% to 60% for HCV-1 infection with a 48-week PegIFN-ribavirin treatment, compared with 76% to 95% for HCV-2 or HCV-3 infections with a 24-week regimen [23, 36, 37, 43, 44, 55, 56]. Patients with HCV-4, which is common in Egypt, are intermediate in responsiveness to therapy between those infected with HCV-1 and HCV-2 or HCV-3, and it is suggested that they should be treated for a full 48 weeks with full-dose ribavirin, like patients with HCV-1 [4, 41]. There is insufficient experience to provide recommendations for the treatment of persons with HCV-5 and HCV-6 so far. Experienced providers need to make treatment judgments on a case-by-case basis. Since HCV genotype is the strongest predictor of responses to IFN-based therapy for CHC, it should be determined in all HCV-infected persons prior to treatment to determine the duration of therapy and the likelihood of response [7].

Pretreatment HCV RNA level, even less important than HCV genotype, is a predictor of sustained response in IFN-based therapy [5, 6, 11, 37, 57]. A higher HCV RNA level predicts a lower response rate. The impact of HCV RNA level on the response to combination therapy was different between patients with different HCV genotype infections. High viral load (with a cutoff value of 200.000 copies/mL, or 40.000 IU/mL) influenced the response rate in patients with HCV-1 (41% versus 56%) but not those in patients with HCV-2 or HCV-3 (74% versus 81%) [36]. Under

the circumstances of a determined HCV genotype for CHC patients, testing HCV RNA levels is beneficial and recommended for HCV-1 patients but seems variable for HCV-2 or HCV-3 patients [7].

HCV viral quasispecies evolution is considered another key element determining treatment response [58]. Higher quasispecies complexity at baseline has been observed in nonresponders than in sustained virological responders [59]. An increasing number of mutations within the carboxyl terminal region of the HCV nonstructural 5A protein, named the IFN-sensitivity-determining region (ISDR), were correlated with treatment response in HCV-1-infected patients [60]. Patients infected with the so-called mutant type, defined by four or more amino acid substitutions in the ISDR, showed a more favorable response toward IFN-based therapy in Japan and Taiwan [60, 61]. However, these findings were not observed in a European study [62]. Additionally, a high degree (≥ 6) of sequence variation in the variable region 3 (V3) plus its upstream flanking region of NS5A (amino acid 2334–2379), referred to as IFN/RBV resistance-determining region (IRRDR), would be a useful marker for predicting SVR, whereas a less diverse (≤ 5) IRRDR sequence predicts non-SVR [63].

5.1.2. Host Factors. The presence of bridging fibrosis and cirrhosis has been reported as one of the most unfavorable predictors for IFN-based therapy [5, 6, 12, 51, 64, 65]. Patients with cirrhosis generally respond poorly to standard IFN monotherapy, with SVR rates of 5% to 20% [6, 32]. Responses are improved when conventional IFNs or PegIFNs are combined with ribavirin, resulting in SVR rates of 33% to 44% [6, 36, 37].

A gender effect on response has been reported. Female sex was a predictor of SVR in studies of conventional IFN-based therapy [51], but not in the studies of PegIFN-ribavirin [11, 36, 43]. Younger patients (<40 years) had higher SVR rates with PegIFN-ribavirin [36, 37, 43]. Sustained responders were younger than nonresponders by an average of 5 years [66].

Several studies have demonstrated that SVR rates are lower in patients with coexistent insulin resistance and/or hepatic steatosis or steatohepatitis [67, 68]. In HCV-1 patients treated with PegIFN-ribavirin, a lower SVR rate was observed in patients with insulin resistance (homeostasis model of assessment, HOMA-IR > 2) compared to those without insulin resistance [69, 70].

CHC patients with body mass indexes $>30\,kg/m^2$ are more likely to be insulin-resistant, to have more advanced hepatic steatosis or steatohepatitis and fibrosis, and to experience a reduced response to combination therapy [71, 72]. Additionally, other possible mechanisms of the impact of obesity on the therapeutic response include the linear correlation of efficacy and body-weight-based doses of ribavirin (10.6–15 mg/kg/day) [37]. To encourage weight loss and exercise before treatment, which has been associated with a reduction in steatosis fibrosis scores, is the most direct approach for formulating more effective treatment regimens [73].

Excessive alcohol use could reduce the likelihood of a response to therapy [74, 75]. To increase the efficacy of antiviral therapy, it has been suggested that abstinence be recommended before and during treatment for CHC [45].

Racial differences in response to efficacy of IFN exist and have been one of the host factors. A lower response rate to IFN monotherapy was observed among African-American patients compared with White patients [17, 76]. A pool analysis of two clinical trials with IFN-ribavirin combination therapy demonstrated that SVRs were highest among Asians (61%), followed by Whites (39%), Hispanics (23%), and African-Americans (14%) [77]. Hispanics and African-Americans were less likely to respond to PegIFN-α-ribavirin compared to Whites [78]. In studies of Taiwanese CHC patients, the SVR rate was 23.7%, 37.1%, and 63.6% for a 24-week treatment of 3 MUs of IFN-α 3 times weekly alone, 6 MUs of 3 times weekly alone, and 3 MUs of 3 times weekly plus ribavirin, respectively [65, 79]. The SVR rate of HCV-1b patients to 24-week PegIFN-α-ribavirin was 48.9% to 65.8% and could be as high as 80% with a 48-week regimen in Taiwan [79, 80]. A relative lower body weight (67–70 kg) in Asian patients compared to U.S. patients (78–81 kg) may also play an important role [71].

The different ethnic response rates may reflect the important role of genetics. Host genetic variations are probably involved in the efficacy of IFN-based therapies for CHC [81]. Genetic polymorphisms of human leukocyte antigen, CC chemokine receptor 5, cytotoxic T lymphocyte antigen-4, interleukin-10, low molecular mass polypeptide 7, MxA, and transforming growth factor-β1 have been reported to have significant associations with responsiveness [82–89]. TNF-α-308 polymorphism was associated with SVRs to IFN-ribavirin in patients with HCV-1b infection and a high viral load [90]. These results reflect the important role of unique genetic predisposition, at least in part, in the response to IFN-based therapy for CHC. Recent advances in pharmacogenomics have demonstrated the potential applications of genetic single nucleotide polymorphism and expression patterns in determining treatment responsiveness in CHC [91, 92]. A recent candidate gene study showed that genetic variation in the *IL28B* gene, which encodes IFN-λ3, is associated with spontaneous HCV clearance [93]. Several genome-wide associated studies observed that IL28B single nucleotide polymorphisms played an important role in the treatment outcome of PegIFN-RBV for CHC [94–96]. A genome-wide association study in 2010 confirmed that IL28B genetic variation was the strongest genetic predictor in both natural and treatment-induced control of HCV. No SNP outside the IL28B/A locus reached genome-wide significance [97]. The increasing evidence for the role of IFN-λ3 for both spontaneous and treatment-induced control of HCV infection opens new avenues for prognosis and treatment of HCV infection. Individuals with HCV genotype 1 or 4 who carry the risk allele, particularly in homozygosis, will have a very low probability of natural or treatment-induced clearance. These individuals would be prime candidates for novel therapeutic strategies [97]. Half of the ethnic differences in response to interferon and ribavirin combination therapy can be explained by genetic polymorphism of IL28B [94].

Because of the presumably shared routes of transmission, approximately one-fourth to one-third of all persons infected with HIV are coinfected with HCV [98]. Patients with HIV-HCV coinfection have been shown to respond less favorably to antiviral therapy than patients infected with HCV alone [98, 99]. Several RCTs recommended 48 weeks of PegIFN-ribavirin for HCV, regardless of HCV genotype, in HCV-HIV coinfected patients [100, 101].

Dual infections of HCV and hepatitis B virus (HBV) are not uncommon and occur in up to 5% of the general population in HCV-endemic areas [102]. Combined chronic hepatitis B and C leads to more severe liver disease and an increased risk of HCV [103]. Although HBV-HCV dual infection was refractory to conventional IFN monotherapy [104], recent studies in Taiwan have demonstrated that conventional IFN-ribavirin combination therapy was effective in HCV clearance among HCV-dominant, HBV/HCV dually infected patients [105, 106]. Recently, a large, open-label, comparative, multicenter study confirmed the efficacy of PegIFN-ribavirin for patients with chronic HCV-HBV dual infection in Taiwan [107].

Nonresponders are more resistant to retreatment with subsequent IFN-based therapy, compared to relapsers (OR = 3.912; 95% CI = 1.459–10.49) [108]. Retreatment with PegIFN-ribavirin could achieve an SVR rate of 47% to 60% for relapsers and 18% to 23% for nonresponders [109–112].

5.2. On-Treatment Predictors and Response-Guided Individualized Therapy. During IFN-α-based therapy, HCV RNA levels generally fall in a biphasic manner [74]. The first rapid phase of viral suppression, from a few hours after the first IFN-α injection to the end of the first day, is related to an inhibition of viral replication by a direct, nonspecific action of IFN-α. This early initial decline in HCV RNA levels correlates poorly with the eventual response to IFN-based therapy [74, 113]. The second, slower phase of viral suppression, beginning on day 2 and gradually leading to seroclearance of HCV RNA, is possibly related to the gradual clearance of infected cells by the patient's immune system, while HCV replication is efficiently inhibited. This phase, less influenced by the dosage of IFN and HCV genotype, exhibits a good response to PegIFN and is an excellent marker of an SVR to the treatment [36, 54, 74].

An RVR at week 4 could predict an SVR to IFN-ribavirin with a high degree of accuracy in both HCV-1 and HCV-2 patients, with positive predictive values of 78% and 92%, respectively [23]. Recent studies have demonstrated that an RVR is the single best predictor of an SVR to PegIFN-ribavirin for HCV-1 [114, 115] and HCV-2 or HCV-3 patients [23, 55, 56, 116]. For HCV-1 or HCV-4 patients with lower baseline viral loads and an RVR, an abbreviated 24-week regimen could achieve a comparable SVR rate with a standard 48-week regimen [115, 117, 118]. Selected patients with RVR might have their treatment courses abbreviated to 16 weeks if they are infected with HCV-2 or HCV-3 [23, 56]. But, the shortening of therapy duration for genotype 2/3 with RVR is still controversial [119]. Abbreviated regimens may be considered in patients with a low baseline viral load who achieve an RVR [120, 121].

Among patients with an EVR, the likelihood of an SVR is only 72% [22]. However, as a negative predictor, non-EVR is even an more robust predictor. In cases without an EVR, the likelihood of an SVR is approximately 0% to 2% [122]. In Taiwan, the non-EVR is a significantly negative predictor in HCV-1 patients, but not in HCV-2 patients [23]. Thus it is recommended that HCV-1 patients who do not achieve an EVR at week 12 should discontinue the therapy beyond 12 weeks [22, 78]. Recently, stratification of early virological response (EVR) into complete EVR (cEVR) and partial EVR (pEVR) has been possible to further improve the prediction of an SVR and may allow for optimizing treatment duration in non-RVR patients [123]. Studies for HCV-1 non-RVR patients have demonstrated that the current recommended 48 weeks of treatment could achieve high SVR rates in patients with a cEVR but could lower rates of SVR in patients with a pEVR [124, 125]. The SVR rates would be more than 90% if patients could reach a cEVR with a standard regimen (48 weeks for HCV-1 or 24 weeks for HCV-2) [41]. For non-RVR patients, HCV viral loads $<10^4$ IU/mL at week 4 provided an early and accurate prediction of who would or would not achieve a cEVR and following SVR [41]. In HCV-1-infected patients with a pEVR, the SVR rates were 10% and 21% only and the relapse rates were up to 83% and 63% in the 24-week and 48-week groups, respectively. The treatment responses were inadequate, even with a standard 48-week regimen in these patients [124, 125].

Based on these predictors associated with treatment efficacy, response-guided individualized therapy has become a major consideration for clinicians. It is desirable to expose CHC patients to the lowest doses and shortest durations of treatment, to reduce the likelihood of adverse events and to minimize costs, without compromising treatment efficacy. On the other hand, some difficult-to-treat patients have to receive longer and/or higher dose therapy to achieve responses. To date, HCV genotype, baseline viral load as well as on-treatment virological responses will provide information for individualized therapy decisionmaking for CHC patients in the near future [115, 126]. People who have an RVR may have a chance to abbreviate their treatment courses to avoid unnecessary costs and preventable drug side effects. In patients without an RVR treated with standard of care, the SVR rate would be more than 90% if cEVR could be accomplished. In patients with only a pEVR, it has been suggested to extend the treatment course to 72 weeks [124, 125, 127] or adhere to high-dose peginterferon plus ribavirin combination therapy [128]. In the future, additional therapy other than interferon-based treatment, such as protease inhibitors, might be anticipated in those difficult-to-treat patients. One would like to be able to evaluate whether a treatment response is likely as early as possible so that individualized strategies can be made or altered earlier before or during the treatment course. HCV viral loads $<10^4$ IU/mL at week 4 provided an accurate prediction of cEVR and SVR in non-RVR patients [41].

Medical adherence is an important factor associated with response to IFN-ribavirin, especially among patients with HCV-1 infection. In a retrospective analysis of data collected in the large registration trials of IFN-ribavirin, SVRs have

been reported to be more likely in patients who had taken at least 80% of all projected IFN injections and at least 80% of all projected ribavirin for at least 80% of the anticipated duration of treatment [39].

6. Protease Inhibitors and IFN-Based Therapy

Recent development of direct-acting antiviral agents, also named "specifically targeted antiviral therapy for hepatitis C" (STAT-C) compounds, to treat HCV has focused predominantly on inhibitors of the viral enzymes NS3/4A protease and the RNA-dependent RNA polymerase NS5B [129, 130]. NS5B polymerase inhibitors in general have a lower antiviral efficacy than protease inhibitors [130]. The administration of HCV NS3/4A protease inhibitors to patients with chronic HCV infections has demonstrated that they have dramatic antiviral effects and that compounds acting via this mechanism are likely to form a key component of future anti-HCV therapy [131]. Newer data have demonstrated promise for 2 protease inhibitors, SCH 503034 (boceprevir) and VX-950 (telaprevir), both of which appear to be able to improve sustained response while shortening duration of therapy [132]. Telaprevir (VX-950), the HCV protease inhibitor, is in the most advanced phase of clinical development [133]. A first case of sustained virological response (SVR) achieved in a patient with chronic hepatitis C by monotherapy with telaprevir without interferon therapy was reported [134]. Owing to a low genetic barrier, resistant variants emerge within a few days when used in monotherapy, thereby decreasing its efficacy. Consequently, telaprevir has been combined with pegylated-interferon and ribavirin in clinical trials. This triple combination is more effective but has a higher rate of adverse events (notably rash) than the standard of care, despite the shorter duration of therapy [133]. Results of the milestone studies PROVE 1 and 2 indicate that 12 weeks of telaprevir-based triple therapy is too short because of the high rate of relapse after treatment completion. However, 24 to 48 weeks of total therapy including 12 weeks of triple therapy with telaprevir in addition to standard treatment greatly improved SVR rates in treatment-naïve genotype 1 patients compared with the standard of care. PROVE 3 has shown that telaprevir is also highly effective in the treatment of prior nonresponders or relapsers infected with HCV genotype 1 [130, 135].

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Hindawi Publishing Corporation
Hepatitis Research and Treatment
Volume 2010, Article ID 562578, 9 pages
doi:10.1155/2010/562578

Review Article

Long-Term Effects of Antiviral Therapy in Patients with Chronic Hepatitis C

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Received 22 June 2010; Accepted 25 July 2010

Academic Editor: Ming-Lung Yu

Chronic hepatitis C is a major cause of chronic liver disease globally, and the natural history of progression may lead to cirrhosis with liver failure, hepatocellular carcinoma, and premature liver-related death. Emerging data demonstrates that interferon-based therapy, particularly among those achieving a sustained virologic response (SVR), is associated with long-term persistence of SVR, improved fibrosis and inflammation scores, reduced incidence of hepatocellular carcinoma, and prolonged life expectancy. This reduction in the rate of progression has also been demonstrated in patients with chronic hepatitis C and cirrhosis in some but not all studies. The majority of these results are reported with standard interferon therapy, and long-term results of peginterferon plus ribavirin therapy with a higher likelihood of SVR should have a yet greater impact on the population of treated patients. The impact on slowing progression is greatest in patients with an SVR, less in relapsers, and equivocal in nonresponders. Thus, the natural history of chronic hepatitis C after completion of antiviral therapy is favorable with achievement of an SVR, although further data are needed to determine the likely incremental impact of peginterferon plus ribavirin, late long-term effects of therapy, and the benefit of treatment in patients with advanced hepatic fibrosis.

1. Introduction

Hepatitis C virus (HCV) is a leading cause of chronic liver disease and a major public health problem, with 130 to 170 million people infected worldwide [1]. Chronic HCV infection may result in serious sequelae, such as end-stage cirrhosis, hepatocellular carcinoma (HCC), need for liver transplantation, and premature death [2]. Treatment of HCV infection with interferon was first used successfully in 1986 [3], and interferon in its pegylated formulation in combination with ribavirin is the preferred therapy for the treatment of patients with acute or chronic HCV infection [4]. The current treatment of chronic hepatitis C is peginterferon alfa-2a or alfa-2b plus ribavirin, with approximately 55% to 65% of patients across all genotypes achieving a sustained virologic response (SVR) [5–7]. However, only 42% to 52% of patients with genotype 1 infection have an SVR after 48 weeks of combination therapy in pivotal trials [5–7]. SVR is traditionally defined as an undetectable serum HCV RNA 24 weeks after the discontinuation of therapy and is regarded as a "cure" [4], although an undetectable HCV RNA 12 weeks after completion of therapy appears to be equally as relevant for prediction of an SVR in patients treated with peginterferon plus ribavirin [8]. The primary goal of treatment of chronic hepatitis C is to prevent late liver-related morbidity and mortality, and measurement of SVR is the short-term surrogate used to predict the long-term efficacy of antiviral therapy. The purpose of this concise paper is to summarize current knowledge on the impact of antiviral therapy on persistence of SVR and long-term outcomes, including impact on hepatic fibrosis, incidence of HCC, and life expectancy. These data provide a rationale for the early use of antiviral therapy not only to achieve an SVR but also to favorably impact the long-term prognosis of chronic hepatitis C.

Table 1: Durability of undetectable serum HCV RNA after SVR.

Author, year of publication (reference)	Patients no.	Followup period years	Detectable HCV RNA no. (%)
Marcellin et al. [9]	80	4 (mean)	3 (4)
Reichard et al. [10]	26	5.4 (mean)	2 (8)
McHutchison et al. [11]	395; 151	5 (mean)	10 (2.5%); 2 (1.3)[1]
Veldt et al. [12]	286	Up to 4.9	12 (4)
Formann et al. [13]	187	2.4 (median)	0 (0)
Desmond et al. [14]	147	2.3 (mean)	1 (0.7)
Lindsay et al. [15]	366	4.8 (mean)	4 (1)
Maylin et al. [16]	344	3.3 (mean)	0 (0)
George et al. [17]	147	5.4 (median)	0 (0); 9 (6)[2]
Kim et al. [18]	73	Not reported	8 (11); 1 (1.4)[3]

Patients in the above studies who were followed after an SVR are heterogeneous and include those with all genotypes and various treatment regimens, including interferon monotherapy, interferon plus ribavirin and peginterferon plus ribavirin, and various treatment durations for 24 or 48 weeks; patients were generally naïve to prior therapy, but relapsers were included in some studies.

[1]395 patients with an SVR and participating in 4 studies were followed, including naïve and relapsed patients, and patients were treated with interferon monotherapy or interferon plus ribavirin for 24 or 48 weeks; of these 395 patients, a subset of 151 patients were naïve and received interferon plus ribavirin for 48 weeks.

[2]No patient had detectable HCV RNA using PCR (sensitivity = 29 IU/mL); 9 patients had HCV RNA detectable by TMA (sensitivity = 5.3 IU/mL) on one sample (mean of 4 samples from the 9 patients), but all other samples of these 9 patients were negative by TMA.

[3]HCV RNA was detectable by qualitative PCR in 8 patients, but only one patient had persistent viremia.

2. Sustained Virological Response Persists Long Term

The earliest study demonstrating that a sustained response to antiviral therapy was associated with long-term biochemical and virological responses as well as histologic improvement was reported by Marcellin and colleagues from France [9]. In this study of 80 patients who had a 6-month sustained biochemical and virologic response, mean followup of 4 years showed that 93% of patients had a persistently normal alanine aminotransferase (ALT) level, and 96% had undetectable serum HCV RNA. A comparison of hepatic histology before and 1 to 6.2 years after completion of interferon therapy showed improvement in 94% of patients, and HCV RNA was undetectable in the liver in 1 to 5 years after treatment in all 27 patients tested. In an analysis of 4 large trials in which 395 patients were followed after achieving an SVR with interferon alfa-2b with or without ribavirin, the actuarial likelihood of maintaining response after a mean 5-year followup was 99% ± 1%, with overall 10 patients (2.5%) developing detectable HCV RNA and all within 2 years of followup [11]. Thus, this analysis of a large study database confirmed that late relapse is rare in patients who remain HCV RNA negative 24 weeks after completion of interferon-based therapy. Multiple other studies [10, 12–18], including one small study with a 10-year mean followup [19], showed that SVR predicts a high likelihood of long-term SVR (Table 1). One of these studies followed 344 patients for a median duration of 3.3 years (range, 0.5 to 18 years) after completion of interferon-based therapy with an SVR, and showed that serum HCV RNA remained undetectable in 1300 samples, indicating that none had a relapse with up to 18 years of followup [16]. It is unclear if late detection of serum HCV RNA after an SVR in a small number of patients represents true relapse, especially if the detection occurs only once or is intermittent and with use of a very sensitive assay. Although additional followup studies may provide further clarification of this distinction, an SVR appears for now to be durable and an accurate reflection of a cure.

3. Natural History of Chronic Hepatitis C

The progression of HCV infection is relatively slow, and the risk of developing cirrhosis ranges from 4%–25% in infected persons over 20–30 years of followup [4, 26–28]. Older age at the time of HCV infection, male gender, obesity, immunosuppression, and heavy alcohol intake are associated with a more rapid progression rate [28–32]. The annual rate of developing HCC in the presence of cirrhosis ranges from 1%–3% in Western countries to 5%–7% in Japan [33–38]. The higher proportion of elderly patients infected with HCV in Japan may explain the higher incidence rate in this country, since older age accelerates the development of HCC [39]. Hepatic decompensation of cirrhosis occurs at a rate of 3% to 4% per year [33, 34].

4. Antiviral Therapy Improves Hepatic Fibrosis and Inflammation

Changes in histologic findings following a course of interferon therapy have been studied extensively. Shiratori and colleagues [40] analyzed paired liver biopsies of 593 patients; 487 received interferon monotherapy for no longer than 6 months and 106 were untreated. Fibrosis regressed at a rate of 0.28 Metavir units per year in those who achieved an SVR. A meta-analysis of data from 1,013 patients from 3 large

randomized trials [41–43] demonstrated that peginterferon treatment reduced inflammation and fibrosis in patients with an SVR or who relapsed, but not in nonresponders [44]. This improvement was more prominent with use of peginterferon rather than interferon. Significant improvement has also been shown after use of peginterferon and ribavirin combination therapy, with achievement of SVR [45]. Even in patients with advanced fibrosis or cirrhosis, inflammation and fibrosis were improved with interferon monotherapy, peginterferon monotherapy, or peginterferon plus ribavirin combination therapy [44–46]. In a pooled set of data from 3,010 naïve hepatitis C patients with pretreatment and posttreatment biopsies form 4 randomized trials of 10 different regimens using combinations of standard interferon alfa-2b, peginterferon alfa-2b and ribavirin, it was shown that there was a 39% to 73% improvement in necrosis and inflammation [45]. In addition, all regimens significantly reduced fibrosis progression rates in comparison to rates before treatment, with the best results noted with peginterferon plus ribavirin. In particular, cirrhosis was reversed in 49% of patients with baseline cirrhosis. Six factors were associated with the absence of significant fibrosis after treatment: baseline fibrosis stage, SVR, age <40 years, body mass index <27 kg/m^2, no or minimal baseline activity, and HCV RNA level <3.5 million copies/mL [45]. In contrast to the study of Cammà et al. [44], the analysis of Poynard and colleagues showed slowing of the natural progression of fibrosis in nonresponders, as well as those with an SVR and relapsers [45]. A limitation of both of these studies is the relatively short time between paired biopsies, that is, before treatment and 24 week after completion of therapy.

In summary, studies of antiviral therapy with interferon monotherapy, peginterferon monotherapy, or peginterferon plus ribavirin combination therapy demonstrate an improvement in inflammation and fibrosis in patients with an SVR, to a lesser extent in relapsers, and uncertain benefit in nonresponders.

5. Antiviral Therapy Is Associated with a Reduced Incidence of Hepatocellular Carcinoma

The Inhibition of Hepatocarcinogenesis by Interferon Therapy (IHIT) Study Group in Japan has been studying the development of HCC in approximately 3,000 Japanese patients with chronic hepatitis C and has demonstrated that interferon therapy reduces the risk of HCC by half including down to one-fifth in biochemical or virological responders, compared with untreated patients [37]. The preventive effect of interferon on development of HCC was also reported by Imai et al. [39], who conducted a retrospective study of 419 patients receiving interferon monotherapy for 6 months and compared the incidence rate of HCC with untreated controls. The relative risk (RR) for HCC was 0.06 ($P = .007$) in patients with an SVR, and 0.51 ($P = .15$) in relapsers, and 0.95 ($P > .2$) in nonresponders, indicating that only an SVR was associated with a reduced risk of HCC. In contrast, another study demonstrated that the incidence of HCC was decreased not only in patients with an SVR, but also in relapsers when compared with nonresponders [47]. Compatible with the studies cited above, the IHIT Study Group has also demonstrated reduced inflammation and fibrosis in patients with an SVR [40]. However, it should be noted that a reduction in the risk of HCC does not necessarily indicate improvement in overall survival, and interferon is less effective in patients with cirrhosis. In addition, cirrhotic patients tend to be older, and liver-unrelated mortality may be significant and obscure any potential benefit of interferon therapy.

6. Life Expectancy Is Prolonged with Interferon-Based Therapy

It would be expected that long-term durability of an SVR, improvement in fibrosis and inflammation, and a reduced incidence of HCC would translate into a prolonged life expectancy. In fact, recent studies with sufficiently long followup are now reporting this ultimate end-point of antiviral therapy [36, 48–50]. In a retrospective cohort study of 7 university hospitals and 1 regional core hospital in Japan, 2,889 patients with biopsies, including 2,430 patients receiving interferon and 459 untreated patients were analyzed [49]. Compared with the general population, overall mortality was high among untreated patients but not among the treated patients. In the interferon-treated patients, the risk of liver-related death was reduced compared with untreated patients, while the risk of liver-unrelated death remained unchanged. In another study of 459 patients followed for a mean of 8.2 years, multivariate regression analysis revealed that interferon treatment decreased the risk ratio for overall death and liver-related death, particularly in patients with an SVR [48]. Once again, interferon showed no association with liver-unrelated death. Another retrospective cohort study of 2,954 patients, including 2,698 who were treated and 256 who were untreated, showed similar results [50]. Over a mean 6-year followup, interferon therapy improved survival in chronic hepatitis C patients showing a biochemical as well as a virological response by preventing liver-related deaths. Finally, a prospective cohort 6.8-year followup study of 345 patients with cirrhosis, of whom 271 were treated and 74 not treated, showed that interferon inhibited the development of HCC and also improved survival [36]. In contrast to the above findings, an Australian study demonstrated that IFN treatment reduced liver complications, but the beneficial effect of achieving an SVR was marginal ($P < .058$) in a multivariate analysis [51].

7. Impact of Antiviral Therapy in Patients with Chronic Hepatitis C and Cirrhosis

What about the impact of antiviral therapy in patients with chronic hepatitis C and cirrhosis? Shiratori et al. [36] conducted a prospective study on 345 cirrhotic patients, of whom 271 received interferon monotherapy for a median of

6.5 months, evaluating the cumulative incidence of HCC and survival relative to 74 untreated patients; SVR was attained in 15% of these patients. In patients who achieved an SVR, the age-adjusted hazard ratio (HR) for developing HCC and death was significantly reduced to 0.31 ($P < .001$) and 0.05 ($P = .003$), respectively, whereas the outcomes for those who did not attain an SVR were not significantly different from the control group. Bruno et al. [52] performed a retrospective cohort study of 920 cirrhotic patients treated with interferon monotherapy for 1 year, which resulted in an SVR rate of 13.5%. Failure to achieve an SVR had a higher risk of liver-related complications, HCC, and liver-related mortality. Thirty-three percent of patients achieved an SVR after peginterferon plus ribavirin combination therapy and had better outcomes than those who did not attain an SVR [53]. Multivariate analysis revealed that a failure to achieve an SVR was associated with a higher risk of liver-related complications, HCC, and liver-related mortality compared to those who achieved an SVR.

However, several studies have demonstrated no beneficial effect of IFN therapy on the prognosis of cirrhotic patients [33, 54, 55]. A lower SVR rate (4%–9%) or shorter followup period (median, 2.1 years) in these studies may have underestimated the benefit of interferon therapy. A recently published meta-analysis demonstrated that antiviral treatment was associated with a reduced risk of HCC in patients who attained an SVR, compared with nonresponders; the best outcomes were seen in patients treated with ribavirin-based regimes, which confirms the results of other meta-analyses [56–58]. The attainment of SVR also demonstrated prevention of the development of esophageal varices [34].

There have been case reports [59] and long-term followup studies that have shown the development of HCC in patients with advanced hepatic fibrosis after the achievement of an SVR [17, 36, 52, 55, 60]. In one followup study, the two patients who developed HCC were diagnosed 5.8 and 7.3 years after having achieved an SVR. These observations underscore the continued risk of HCC and need for ongoing surveillance with imaging and alpha-fetoprotein testing in patients with chronic hepatitis C and advanced hepatic fibrosis, even after an SVR.

In summary, multiple studies have shown that the achievement of an SVR with antiviral therapy reduces, but does not eliminate, the incidence of HCC and decreases liver-related complications and liver-related mortality in patients with chronic hepatitis C, and in some studies also in cirrhotics. However, the effect of antiviral therapy is controversial in patients who were responders during treatment and subsequently relapsed after treatment and probably has limited benefit in nonresponders.

8. Preventive Effect of Interferon on Recurrence of Tumor after Treatment of HCC

To evaluate effects of interferon treatment on HCC recurrence after resection of HCC, studies were performed in which patients were randomized to receive interferon treatment or to remain untreated [61, 62]. Patients in the interferon-treated group received interferon alpha for approximately 2 years, but only 2 patients (13%) achieved an SVR. HCC recurrence was observed in 33% of the interferon-treated group and in 80% of the control group. The recurrence rate was significantly lower and the cumulative survival rate significantly higher in the interferon group than in the control group [61, 62]. In another randomized controlled study, 74 patients with cirrhosis and low HCV RNA loads were assigned to receive interferon for 1 year ($n = 49$) or to an untreated control group ($n = 25$) after complete ablation of HCC by percutaneous ethanol injection therapy [63]. SVR was achieved in 29% of patients. Interferon treatment seemed to suppress the rate of a second or third recurrence of HCC, but not a first recurrence, and improve the survival rate. Several studies, including one meta-analysis with one exception, indicate that the achievement of SVR appears to prevent the recurrence of HCC [64–69]. However, as virtually all of these studies were conducted in Japan, a definitive conclusion should await further reports from other regions of the world.

9. Impact of Maintenance Therapy on Outcomes of Chronic Hepatitis C with Advanced Fibrosis

An earlier randomized controlled trial revealed that long-term low-dose interferon treatment for patients who did not obtain an SVR with previous standard interferon-based therapy improved liver histology [20] (Table 2). In addition, Nishiguchi et al. [70, 71] demonstrated that interferon monotherapy for 12 to 24 weeks reduced the occurrence of HCC and disease progression. Although these studies included a small number of patients, the results encouraged clinicians to perform large studies to confirm the impact of maintenance therapy for chronic hepatitis C with advanced hepatic fibrosis.

The HALT-C study enrolled 1,050 patients with bridging fibrosis or cirrhosis (defined as an Ishak fibrosis score of 3 or more) who had not responded to a previous 6- or 12-month course of therapy (lead-in phase) with peginterferon and ribavirin [22, 72]. These patients were randomized to receive either no therapy or peginterferon alfa-2a at a dose of 90 μg per week for 3.5 years. Although serum aminotransferase levels, serum HCV RNA levels, and necroinflammatory scores decreased significantly with therapy, the rate of primary outcome (death, HCC, hepatic decompensation, or an increase in the Ishak score of 2 or more points) was similar in the treatment and control groups (34.1% and 33.8%, respectively), confirming the results of a previously reported randomized, controlled trial with a relatively small number of patients [21].

In the HALT-C study, viral suppression of $\geq 4 \log_{10}$ during the lead-in phase of therapy was associated with a significant reduction in clinical outcomes, although a significant difference was not observed between the treatment and control groups [73]. Unexpectedly, viral suppression of $\geq 4 \log_{10}$ during the phase of maintenance therapy did not

Table 2: Randomized, controlled trials to evaluate the effect of maintenance therapy on the progression of HCV-related chronic liver diseases.

Author (study name) [reference number]	Year	Patients	Patient no. (treatment/ control)	IFN regimen	Treatment duration (year)	Control	Preventing effect				
							Liver histology		Disease progression	HCC incidence	Compensation-free survival
							Inflammation	Fibrosis			
Shiffman et al. [20]	1999	Chronic hepatitis (a)	27/26	IFN alfa-2b 3 MU 3 times/wk	2	observation	Yes	Yes	N/A (b)	N/A	N/A
Fartoux et al. [21]	2007	Cirrhosis (c)	51/51	IFN alfa-2a 3 MU 3 times/wk	2	observation	N/A	N/A	No	No	No
Di Bisceglie et al. [22] (HALT-C)	2008	Bridging fibrosis or cirrhosis (d)	517/533	PegIFN alfa-2a 90 μg/wk	3.5	observation	Yes	No	No	No	No
Cardenas et al. [23] and Afdhal et al. [24] (COPILOT)	2009 (e)	Advanced fibrosis or cirrhosis (f)	282/266	PegIFN alfa-2b 0.5 μg/kg/wk	4	Colchicines 1.2 mg/day	N/A	N/A	Yes (reduced variceal bleeding)	No	No (g)
Bruix et al. (EPIC^3) [25]	2009 (e)	Cirrhosis (h)	631 (total)	PegIFN alfa-2b 0.5 μg/kg/wk	3	observation	N/A	N/A	No (i)	N/A	No

(a) The patients of both arms received 6-month course of IFN monotherapy before randomization.
(b) N/A = not available.
(c) 36% and 44% of patients previously failed to respond to IFN monotherapy and IFN/ribavirin combination therapy, respectively.
(d) Patients did not have response to 6- or 12-month lead-in phase peginterferon plus ribavirin therapy.
(e) Only abstract is available.
(f) Patients did not have response to the previous therapy.
(g) Event-free survival was better only in patients with portal hypertension.
(h) Patients did not have response to the previous interferon plus ribavirin therapy.
(i) Clinical events were observed less frequently in the treated group in patients with baseline esophageal varices.

result in an improvement in clinical outcomes. A decrease in serum HCV RNA levels might improve the clinical outcome, even if short-term and transient; thus viral suppression during the lead-in phase may have obscured the beneficial effect of maintenance therapy. The Nishiguchi study tested interferon-naïve patients, which may have accentuated the beneficial effects of interferon [70]. Sixteen percent of treated patients achieved an SVR in this study, which is unlikely in studies enrolling previous nonresponders.

The results from two other large randomized trials are currently unpublished. In the COPILOT study, patients with an Ishak fibrosis score of 3 or more who were nonresponders to interferon therapy were randomized to receive either peginterferon alfa-2b, at a dose of 0.5 μg/kg weekly, or colchicine. This maintenance therapy significantly retarded the development of varies and prevented variceal bleeding, but did not affect overall outcome [23, 24]. The EPIC^3 study also revealed that maintenance therapy was not superior to control except in reducing clinical events in patients with esophageal varices [25]. Finally, a recent meta-analysis assessing the role of the maintenance interferon therapy in nonresponders did not reduce the incidence of HCC (RR: 0.58; 95% CI: 0.33–1.03) [56].

In summary, there are insufficient data to recommend maintenance therapy for patients who did not respond to previous interferon treatment. In the HALT-C study, 3.5 years of low-dose peginterferon improved histologic necroinflammatory scores, but did not reduce the rate of disease progression. Liver histology, especially fibrosis, is closely associated with prognosis [37, 48, 74, 75]. Therefore, further followup of patients in the HALT-C trial may confirm the inhibitory effect of the maintenance therapy on the progression of liver disease.

10. Conclusions

The long-term benefit of antiviral therapy, including reduction in hepatic fibrosis, lower incidence of HCC, and prolonged life expectancy, appears to be limited primarily to patients able to achieve an SVR. A recent systematic review showed that health-related quality-of-life was also improved and that antiviral treatment is reasonably cost effective in treatment-naïve patients as well as relapsers and nonresponders [76]. Therefore, clinicians should aim to treat with antiviral therapy, with the goal of achieving an SVR early in the natural history of chronic hepatitis C. Direct acting antiviral (DAA) agents are expected to provide new treatment options for management of chronic hepatitis C in the near future. Telaprevir, an HCV NS3 protease inhibitor, in combination with peginterferon and ribavirin, induced SVR in approximately 70% of treatment-naïve genotype 1 patients and in 51% of patients who failed previous peginterferon plus ribavirin therapy [77–79]. The increase in SVR rate using DAA agents may provide a long-term benefit to a wider variety of patients, including nonresponders to peginterferon and ribavirin therapy, and further improve the long-term outcomes of therapy including slowed fibrosis progression, reduced incidence of HCC, and prolonged life expectancy.

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Hindawi Publishing Corporation
Hepatitis Research and Treatment
Volume 2010, Article ID 703602, 7 pages
doi:10.1155/2010/703602

Review Article

Predictors of Virological Response to a Combination Therapy with Pegylated Interferon Plus Ribavirin Including Virus and Host Factors

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Received 25 April 2010; Revised 29 June 2010; Accepted 19 July 2010

Academic Editor: Ming-Lung Yu

A combination therapy with pegylated interferon (PEG-IFN) plus ribavirin (RBV) has made it possible to achieve a sustained virological response (SVR) of 50% in refractory cases with genotype 1b and high levels of plasma HCVRNA. Several factors including virus mutation and host factors such as age, gender, fibrosis of the liver, lipid metabolism, innate immunity, and single nucleotide polymorphism (SNPs) are reported to be correlated to therapeutic effects. However, it is difficult to determine which factor is the most important predictor for an individual patient. Data mining analysis is useful for combining all these together to predict the therapeutic effects. It is important to analyze blood tests and to predict therapeutic effects prior to initiating treatment. Since new anti-HCV agents are under development, it will be necessary in the future to select the patients who have a high possibility of achieving SVR if treatment is performed with standard regimen.

1. Progress in Virological Response in the Difficult-to-Treat Patients with Genotype 1 Hepatitis C Virus (HCV) Infection and Factors Correlated to the Efficacy

Recently, the average age of the patients with chronic hepatitis C has been increasing in Japan. Incidence of hepatocellular carcinoma (HCC) in the elderly patients with chronic hepatitis C (65 years or older) has demonstrated to be higher than younger ones when adjusted by the stage of hepatic fibrosis [1]. In Japan, refractory cases with genotype 1b and high HCVRNA levels are seen in as high as 70 percent of chronic hepatitis C patients. The outcome of conventional IFN monotherapy has been an SVR response of 3%–5% after 6 months of treatment in genotype 1b and high HCVRNA patients [2, 3], and virus mutation such as interferon sensitivity-determining region (ISDR) is shown to be correlated with the virological response [2]. The association of ISDR mutations and virological response to IFN monotherapy was denied in an Italian study [4]; however, it was confirmed by a Chinese study [5] and an international meta-analysis [6].

However, pegylated IFN (PEG-IFN) extends the duration of therapy and reduces adverse effects, and for this reason, PEG-IFN has become the cornerstone of therapy. Furthermore, by the combination therapy with PEG-IFN and ribavirin (RBV), the rate of SVR has dramatically improved. Even in the patients with genotype 1b and high HCVRNA level, SVR rate reaches as high as 40%–50%, thereby improving the therapeutic effects both in Western countries [7, 8] and in Japan [9, 10].

It is important to predict the rate of achieving SVR in the individual patient, before initiating treatment. Both virus- and host-related elements have been reported as factors correlated to therapeutic effects of combination therapy [11–13]. A particular focus has been placed on virus mutations, age, gender, fibrosis of the liver, lipid metabolism, and degree of fatty metamorphosis of the liver.

Among these factors related to PEG-IFN and RBV, innate immunity has been shown to be correlated in virological

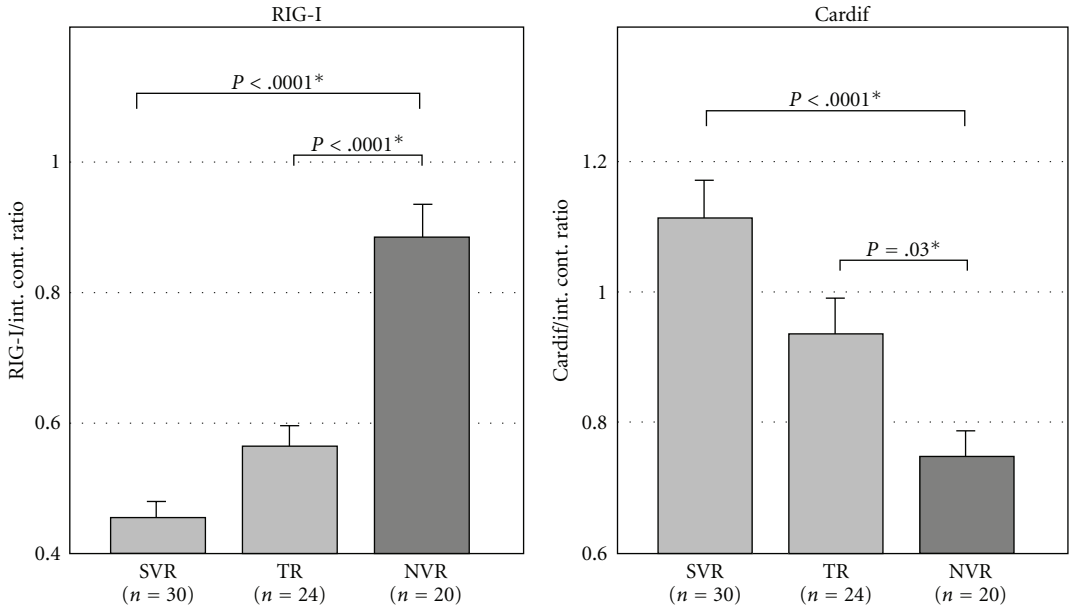

Figure 1: Expression of genes correlated to the intrahepatic innate immunity and virological response to PEG-IFN alpha-2b and RBV combination therapy. Open column indicates SVR ($n = 30$), gray column indicates TR ($n = 24$), and closed column indicates NVR ($n = 20$). Error bars indicate the standard error. The P values were analyzed by the Kruskal-Wallis test. Expression of Rig-I was significantly higher in NVR than in SVR patients, and Cardif expression was higher in SVR than in NVR. The figure was cited from [8].

response. Asahina et al. reported that liver biopsies were performed before the PEG-IFN and RBV combination therapy to examine the correlation between the gene expression involved in innate immunity and the therapeutic effects, and in the patients in whom RIG-I expression is high and the expression of Cardif, an adaptor gene, is low, it was found that there are many nonresponders (NVRs) in which HCVRNA does not become negative during the course of treatment [13]. It was therefore revealed that there are many NVRs among the patients in whom the ratio of RIG-I to Cardif in liver tissue is high and that this ratio is low in the SVR patients. Based on these findings, it has become clear that innate immunity is correlated to therapeutic effects (Figure 1).

Furthermore, it was recently discovered that a single nucleotide polymorphism (SNP) of the host gene IL28B is significantly involved in the therapeutic response to the PEG-IFN and RBV combination therapy [14, 15]. The possibility of becoming an NVR is high in cases of the minor allele carriers of IL28B. However, it is not possible to routinely measure an SNP of IL28B in the clinical setting. In this paper, factors which can actually be used in real clinical practice are discussed for the prediction of the efficacy of PEG-IFN and RBV combination therapy.

2. Amount of HCVRNA

In the patients with chronic hepatitis C, it is not possible to directly measure the amount of virus, and the amount of HCVRNA is measured instead. Currently, a real-time PCR method which has an advantage of wide range and high sensitivity is utilized, and measurements can be taken from a single blood sample of a very small amount, that is, 1.2 log copies/ml, to a very large amount, that is, 8 log copies/ml. This method has a higher level of sensitivity than the conventional Amplicor monitor test and can therefore detect HCVRNA even if only a very small amount exists in the plasma. If the amount of HCVRNA in plasma is less than the range of sensitivity of the real-time PCR method, it is recorded as undetectable level. If the indication is "less than 1.2 log copies/ml of HCVRNA", it means that a very small amount of HCVRNA exists in the plasma. Since the indication of the real-time PCR method is based on log counts, a decrease of 1.0 in the numerical value means that the amount of HCVRNA has decreased to 1/10. With the application of this real-time PCR method, it has become possible to measure amounts of HCVRNA up to 8 log copies/ml, and it has also become possible to predict the efficacy before treatment and to monitor appropriately the reactivity during the course of treatment. However, in the patients in whom a PEG-IFN and RBV combination therapy is performed, SVR can be acquired even when the amount of virus prior to the treatment is quite large. It is therefore difficult to predict the virological response solely from the amount of HCVRNA before starting the treatment. Once treatment has commenced, at what week HCVRNA becomes negative is important for the prediction of therapeutic effects, and this serves as a parameter for deciding the duration of treatment [16].

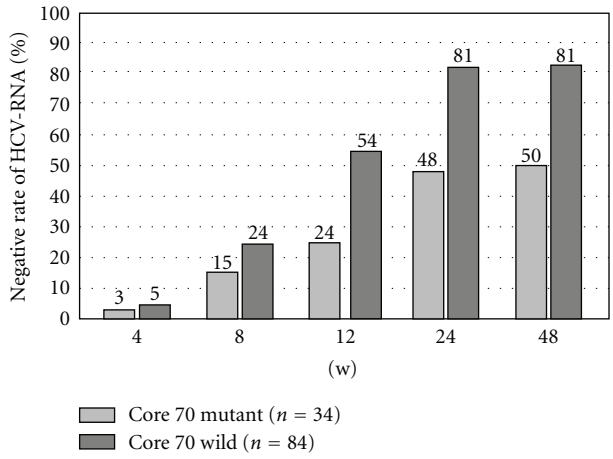

Figure 2: Comparison of aa70 mutations in the HCV core region and the rate of HCVRNA becoming negative during the course of treatment. Compared with the wild type, among the patients of aa70 mutations, there were fewer patients in whom HCVRNA had become negative during the course of treatment.

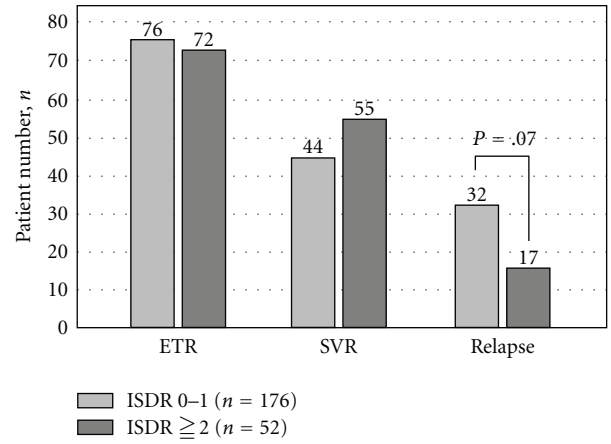

Figure 3: Number of ISDR substitutions and the comparison of virological response, SVR, and relapse at the end of the treatment. Compared with the patients with 2 or more sites of substitutions, the rate of SVR was lower and the rate of relapse was higher in the patients in whom there were fewer substitutions in ISDR, that is, 0 or 1 sites.

Measuring the rate of viral clearance from serum is helpful for predicting the likelihood of a response to PGEIFN and RBV and useful for determining the optimal duration of therapy if the patients start to receive the treatment [17]. In the AASLD practice guideline, response-guided therapy is highly recommended [18]. In two nationwide registration trials conducted in Japan [9, 10], the SVR rate was high, from 76% to 100%, in patients whose HCVRNA was cleared rapidly from serum by week 4 (rapid virological response; RVR), and 71% to 73% in patients who achieved undetectable HCVRNA from week 5 to week 12 (early virological response; EVR). In contrast, the SVR rate in patients with late clearance of HCVRNA from week 13 to week 24 was low at 29% to 36%. No patients without clearance of HCVRNA by week 24 achieved SVR.

The strategy of extending therapy in patients with delayed virological responses, defined as clearance of HCV-RNA between weeks 12 and 24, was evaluated in five studies [19–23]. These results cannot be compared directly with each other because of the heterogeneous study populations, differences in the baseline characteristics, and the different regimens utilized amongst them. Nevertheless, the results showed a trend toward a higher SVR rate by extending therapy from 48 to 72 weeks in patients with delayed virological response.

3. Viral Mutations in Core and NS5A Region

In the patients with genotype 1b HCV infection, the mutations in aa70 and aa91 in the core region have been shown to correlate with early virological response (EVR) during PEG-IFN and RBV combination treatment [11]. If aa70 in the core region is mutated to anything other than arginine and aa91 to anything other than leucine, it is difficult to achieve EVR, and it is thus highly possible that such cases will become nonresponders. The examination results at our institution including 292 patients with genotype 1b infection demonstrated that, in the cases with mutations in aa70 in the core region, the rate of HCVRNA becoming negative during the course of combination treatment was low compared to the wild type of aa70 (Figure 2). However, core aa70 mutation is shown to have quasispecies detectable by cloning, and 70Q clone was positively selected during combination treatment with PEGIFN and RBV [24].

Furthermore, Enomoto et al. reported that the patients with 4 or more amino acid mutations were observed in interferon sensitivity-determining region (ISDR) within NS5A region [2]; SVR rate is higher than 90% by IFN monotherapy, and SVR is less than 10% in the patients with no mutation in ISDR. It has also been reported that, in PEG-IFN and RBV combination therapy, the number of ISDR mutations is involved in the SVR [12].

We analyzed the relationship between virological response and ISDR mutations in the patients with genotype 1b infection treated by PEG-IFN alpha-2b and BRV combination therapy. In the patients with 0 or 1 ISDR mutation, even if the rate of HCVRNA becoming negative at the end of treatment was the same, the rate of SVR would be lower compared with the patients having 2 or more mutations (Figure 3). This demonstrates that there is a higher incidence of relapse after the end of treatment in the patients with 0 or 1 ISDR mutation.

Enomoto and Maekawa reported that mutations both in NS5A-ISDR (interferon sensitivity-determining region) and core 70Q substitution are associated with no early viral response during PEGIFN and RBV combination therapy [25]. Association of core aa70 substitution and mutations in NS5A region is confirmed to be associated with viral response by PEGIFN and RBV combination therapy in a Japanese multicenter cooperative study [26]. The number of

mutations in the interferon sensitivity-determining region was shown to be associated with the viral response to PEGIFN and RBV combination treatment not only in Japan [27], but also in Tunisia [28].

Recently, a consensus has been established that mutations in aa70 in the core region are important for the prediction of HCVRNA becoming negative during the early course of treatment, and the number of ISDR mutations is important for the prediction of relapse after the end of treatment.

4. Adherence

It has been confirmed that it is important to ensure 80% or more of the scheduled dose of both PEG-IFN and BBV in order to improve the rate of SVR, and together with the duration of treatment, the 80 · 80 · 80 rule has been established. However, Schiffman et al. recently reported that the dose of PEG-IFN in the initial stage of administration is important and that, if a sufficient dose of PEG-IFN is administered, then 60% or more of the RVB dose would be enough [29]. It is therefore of primary importance to ensure the dose of PEG-IFN.

In Japan, the average age of patients with chronic hepatitis C is increasing, and achieving good adherence is difficult in many patients. Consequently, the rate of SVR is low in elderly patients. How to improve the rate of SVR in elderly patients is an important issue. With regard to the dose of RBV, reducing the RBV dose based on the calculation of the total body clearance (CL/F) has been proposed to be useful for decreasing the discontinuation and improving the rate of SVR. Although there is no consensus on an appropriate dose of PEG-IFN in elderly patients, if the initial dose is set lower than the usual dose, discontinuation would be reduced. Thus, it is necessary to investigate whether such an initial dose would improve the rate of SVR.

Recently, the risk of hemolytic anemia was clearly demonstrated to correlate with ITAP gene SNP [30]. The predictive implication should be analyzed prospectively in clinical practice.

5. Host Factors

Zeuzem et al. described the factors related to the less response to interferon-based therapy, and he showed that several host factors such as older age, race, and obesity are responsible factors for the poor response to IFN [31]. Recently, insulin resistance which was examined by homeostasis model assessment index (HOMA-IR) was shown to be associated with a lower rate of SVR, and body mass index (BMI) was not identified as a significant factor for the poor response to PEGIFN and ribavirin combination therapy [32]. Insulin resistance was confirmed as a related factor to the nonresponse to interferon-based treatment [33]. However, Charlton et al. reported that obesity itself is an associated factor for decreased efficacy of interferon-based therapies, and they discussed the possible mechanism [34], and obesity was shown to be associated with the increased enhancement of suppressor of cytokine signaling (SOCS) family in the hepatocytes [35].

6. Data Mining Analysis

Both virus- and host-related factors are correlated to therapeutic effects of PEG-IFN and RBV. One important question is which of these factors should be focused on in order to predict the therapeutic effects in an individual patient. In addition, in each individual patient, the host and virological factors are different. It is therefore difficult to predict the virological response in each case before treatment. Furthermore, although it is important to predict the relapse rate when HCVRNA becomes within an undetectable level in an individual patient, prediction of the rate of SVR including virological and host factors and adherence to the treatment has never been carried out in an individual patient.

A data mining analysis is the process of analyzing a large amount of data by a computer in order to develop an algorithm. Conventional statics have been used to examine certain hypothesis. Data mining is superior in that it can set an algorithm, using a computer, based on a large amount of data without a hypothesis.

We therefore conducted at our institute a data mining analysis of the patients with genotype 1b infection having high levels of HCVRNA to whom a PEG-IFN alpha-2b and RBV combination therapy was administered to investigate whether by the 12th week after the commencement of treatment HCVRNA became negative (EVR) (Figure 4) [36]. The most important factor for the prediction of EVR was the steatosis of the liver: when steatosis was observed in 30% or more of hepatocytes, EVR was found to be difficult to achieve. In the patients in whom steatosis was not severe, the second most important factor was the serum LDL cholesterol value. While the rate of EVR was 57% in the patients in whom this value was 100 mg/ml or above, the rate of EVR was 32% in the patients in whom the LDL cholesterol was less than 100 mg/dl.

The higher the LDL cholesterol value, the earlier the HCVRNA became negative. Among the patients with low LDL cholesterol values, while the rate of EVR was 15% in patients 60 years of age or older, the rate was high in the patients under the age of 60 years old, that is, 49%. Among patients under the age of 60, the rate of EVR was low, that is, 31%, in patients with a blood glucose level of 120 mg/dl or above whereas EVR was achieved in 71% of the patients with a blood glucose level of less than 120 mg/dl (Figure 4).

On the other hand, in the patients with high LDL cholesterol values, the next most important factor was age. While the rate of EVR was 50% in patients 50 years of age or older, EVR was achieved in 77% of the patients under the age of 50. Among patients of 50 years of age or older, the next most important factor was the gamma GTP value. While the rate of EVR was 35% in the patients in whom gamma GTP was 40 IU/L or above, EVR was achieved in 60% of the patients where the value was less than 40 IU/L.

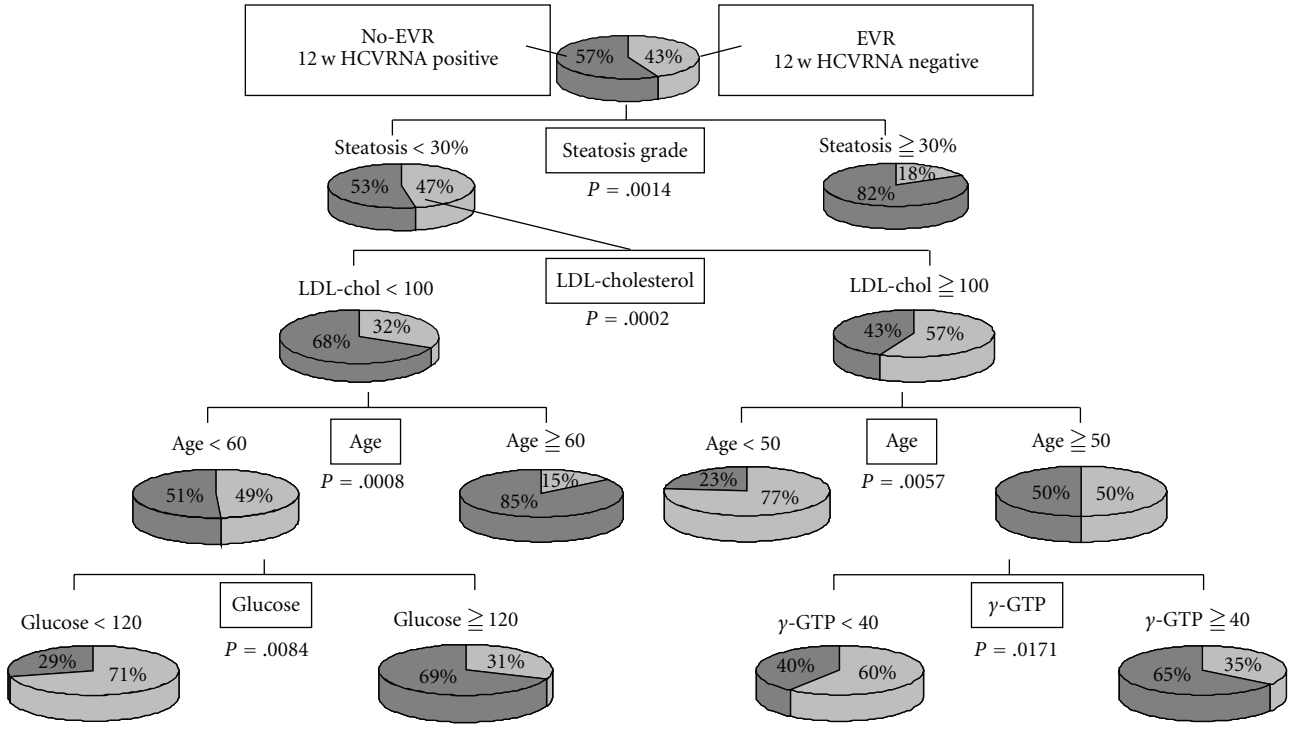

Figure 4: HCVRNA negative (EVR) algorithm at 12th week from data mining analysis of PEG-IFN alpha-2b plus RBV combination in the genotype 1b and high levels of HCVRNA. Both virological and host factors were evaluated by data mining analysis software from SPSS. This figure was cited from [36].

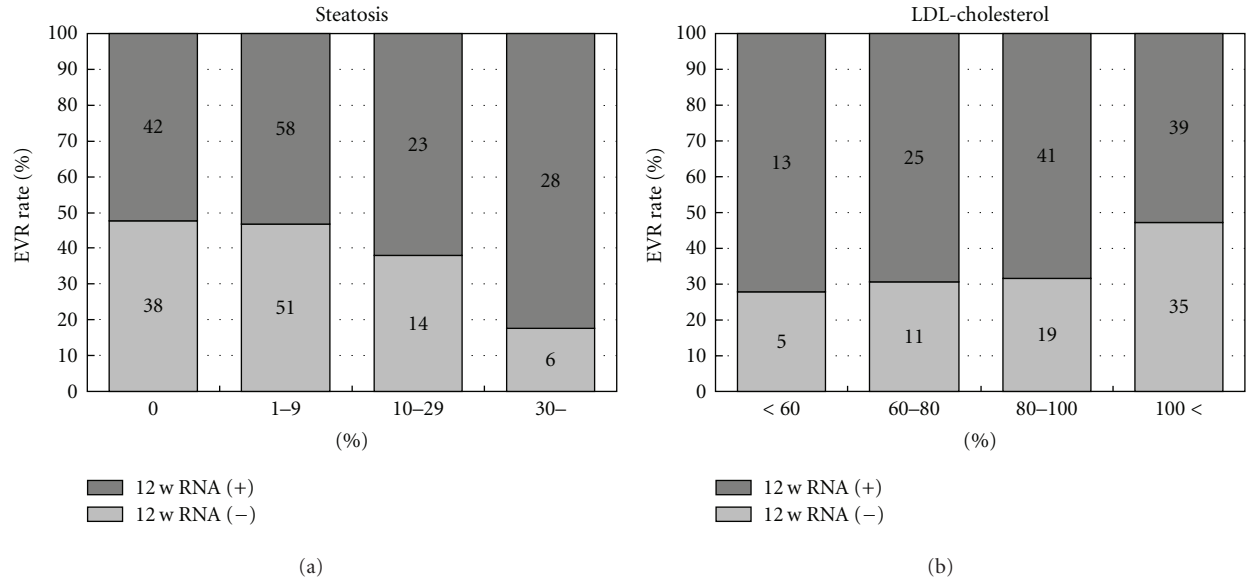

Figure 5: The rate of EVR in the patients with genotype 1b and high levels of HCVRNA, based on fatty deposition in the liver (a), and the LDL cholesterol value (b), respectively. EVR was highly achieved in the patients with less steatosis in the liver, and in those with high serum LDL-cholesterol levels. This is univariate analysis, and cited from [36].

We therefore compared these factors based on the original data. A univariate comparison of the fatty infiltration of the liver and the rate of EVR demonstrated that the rate of EVR was higher when the steatosis of the liver was less severe (Figure 5(a)). In addition, a comparison of the LDL cholesterol value and the rate of EVR demonstrated a significant correlation, confirming that the higher the LDL cholesterol value, the higher the rate of EVR (Figure 5(b)). Therefore, it was also proposed by the results of univariate analysis of each factor extracted from the data mining analysis that these factors were correlated to the rate of EVR.

From these observations, it is likely to improve the viral response to PEGIFN and ribavirin by reducing steatosis of the liver through daily walking or abstinating alcohol intake or by refraining from high-fat diet.

By utilizing data mining, it is therefore possible to assess virus- and host-related factors together and to predict the virological response in each patient, and thereby clinically useful information can be obtained. The algorithm should be validated including a large number of the patients.

Acknowledgment

This paper was supported by a grant from the Japanese Ministry of Health, Welfare and Labor.

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Hindawi Publishing Corporation
Hepatitis Research and Treatment
Volume 2010, Article ID 429243, 10 pages
doi:10.1155/2010/429243

Clinical Study

Tumor Necrosis Factor Receptor 1 Expression Is Upregulated in Dendritic Cells in Patients with Chronic HCV Who Respond to Therapy

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Received 25 March 2010; Revised 28 May 2010; Accepted 31 May 2010

Academic Editor: Tatehiro Kagawa

The present studies assessed the level of tumor necrosis factor receptor (TNFR) expression in peripheral blood mononuclear cells (PBMCs) subsets from patients with chronic HCV undergoing interferon α/ribavirin-based therapy (Ifn/R). *Methods*. TNFR family member mRNA expression was determined using quantitative real-time PCR assays (RTPCRs) in PBMC from 39 HCV+ patients and 21 control HCV− patients. Further subset analysis of HCV + patients (untreated (U), sustained virological responders (SVR), and nonresponders (NR)/relapsers (Rel)) PBMC was performed via staining with anti-CD123, anti-CD33, anti-TNFR1 or via RTPCR for TNFR1 mRNA. *Results*. A similar level of TNFR1 mRNA in PBMC from untreated HCV+ genotype 1 patients and controls was noted. TNFR1 and TNFR2 mRNA levels in PBMC from HCV+ patients with SVR were statistically different than levels in HCV(−) patients. A significant difference was noted between the peak values of TNFR1 of the CD123+ PBMC isolated from SVR and the NR/Rel. *Conclusion*. Upregulation of TNFR1 expression, occurring in a specific subset of CD123+ dendritic cells, appeared in HCV+ patients with SVR.

1. Introduction

Unlike infections by other human hepatitis viruses, hepatitis C virus (HCV) infection of healthy immunocompetent adults is persistent or prolonged in the majority of cases, unlike hepatitis B, where it persist in the minority of cases. HCV appears to have evolved strategies to evade the human immune responses. Investigators have suggested that viral interactions with tumor necrosis factor/tumor necrosis factor receptor (TNF/TNFR) family members may be important in immune evasion. Investigators have reported that HCV core protein binds to the cytoplasmic domain of TNFR1, specifically the "death domain," which may hinder or promote cell death during HCV infection [1]. In addition, the HCV core protein binds to another TNFR family member, lymphotoxin β receptor's (LTβR) intracellular domain, which is involved in signal transduction [2]. Investigators have also demonstrated that the HCV core protein potentiates NF6B activation initiated by TNFα/TNFR and LT$\alpha_1\beta_2$/LTβR interactions, which, in turn may contribute to the chronically activated, persistent state of HCV-infected cells [3, 4]. Interference with signaling through members of the TNF family of receptors may affect the chronicity of HCV via affecting either cell death and/or activation of NFκB [1, 3, 4].

Of interest, other investigators have examined the relationship between HCV and the soluble TNF receptors (*s*TNFR) in the blood. These sTNFR levels appear to peak 9 hours after the first IFNα administration correlating with IFNα serum levels [5]. Other investigators have noted higher levels of TNF and *s*TNFRs in HCV+ patients than in HCV (−) controls prior to treatment and postulated that high

levels of *s*TNFR might modify host responses but found no correlation between levels and response to therapy [6].

TNF receptors are present on the surface of a number of cell subsets and play a role in a variety of functions. For example, TNF/TNFR interactions are imperative for the optimal proliferation and effector functions of CD8 + T cells [7, 8], whose antiviral effects are essential for the clearance of noncytopathic viruses, such as HCV. In addition, LTβR-LIGHT/LT$\alpha_1\beta_2$ interactions have been implicated in both optimal growth and effector functions as well as costimulation of CD8+ T cells [9]. Similarly, TNFR is expressed on macrophages, peripheral blood monocytes, and antigen presenting cells (APCs), cells known to be important in viral infections. Recently, HCV negative strand RNA has been noted in the macrophages of 67% of sustained virological responders to interferon-based therapies [10]. Others have noted the importance of monocytes and dendritic cells in the clearance of HCV [11–14]. The role of TNFR in HCV viral clearance may involve any of these cell subsets.

The largely chronic nature of HCV infection has been attributed to an attenuated antiviral T-cell response. It has been postulated that APC's may become dysfunctional in some way during HCV infection contributing to this attenuation. Specifically, large deficits in IFNα production in plasmacytoid dendritic cells (PDCs) in HCV-infected patients have been reported in [15]. In the same study HCV-infected PDC displayed distinct immunophenotypic features including an increased ability to stimulate a mixed lymphocyte response (MLR) but lower HLA-DR and CD86 expression. This profile suggested that HCV-infected PDC were at an immature stage of differentiation [15].

The present studies were designed to quantify the mRNA and protein levels of the TNF receptor family members, including TNFR1 and TNFR2, in PBMCs during IFNα-based therapy of HCV+ patients. The results of these studies reveal increased peak levels of TNFR1 mRNA levels in responders to IFN-based therapies. Furthermore, by flow cytometric studies and western blot analysis, upregulation of TNFR1 protein expression was noted in specific PBMC subsets. The increase in TNFR1 expression was specifically isolated in PDC (CD11− and CD123^{high}) of HCV-infected patients who responded to IFNα based therapies compared to control patients and nonresponders.

2. Methods

2.1. Patients. Veteran patients >18 years of age with chronic HCV were recruited to IFNα based treatment trials conducted at the Dallas Veterans Affairs Medical Center from February 2002 through July 2005. Thirty-seven patients with genotype 1 consented per institutional review board (IRB) guidelines for blood draws at one or more time points, including 0, 4, 8, 12, 24, and 48 weeks of therapy, where the standard therapy (48 weeks of weight-based dosing of 1.5 μg/kg of pegylated interferon α-2b and 13.5 mg/kg of (ribavirin) for genotype 1 patients was utilized. Thirteen patients were sustained virological responders (SVR, HCV RNA negative 24 weeks after end of therapy), 20 patients were nonresponders (NR, persistently HCV RNA positive during therapy), and 4 patients were relapsers (Rel, HCV RNA negative during therapy but HCV RNA positive after end of therapy). None of the patients analyzed had received interferon prior to the initial blood draw. Similarly, 10 HCV(−) patients and 11 patients with alcoholic liver disease (ALD) or nonalcoholic fatty liver disease (NAFLD) consented per IRB guidelines for a blood draw.

In a second set of patients, samples from 13 genotype 1 HCV+ patients (5 SVR, 6 NR/Rel and 2 HCV+ prior to therapy) and 2 HCV(−) control patients obtained during therapy at <12, 16–24, or 25–44 weeks were collected for a specialized cell subset analysis.

2.2. RNA Isolation and Real-Time PCR Primers. RNA was isolated from PBMC's at 0, 4, 8, 12, 24, and 48 weeks of interferon/ribavirin (Ifn/R) treatment trials from 39 HCV+ men, 10 age- and sex- matched HCV(−) controls, and 11 age- and sex- matched controls with either alcoholic liver disease (ALD) or nonalcoholic fatty liver disease (NAFLD). Initially, 40–60 cc of blood was obtained from HCV+ patients and controls. PBMCs were then separated by density gradient centrifugation and then stored in TRIzol reagent to stabilize the RNA. RNA was then extracted, precipitated, and subjected to DNase treatment, and then the treated RNA underwent reverse transcription.

In order to assess whether there were differences in any specific cytokine receptors or TNF receptor family members, 101 cDNA samples were utilized in a quantitative real time PCR-based assay assessing the relative quantities of cyclophilin, NGFR, CD30, TNFR1, TNFR2, LTβR, IL-2Rα, CD27, FAS, and CD3. RNA from PDC (CD11− and CD123^{high}) and myeloid dendritic cells (MDC) (CD11+ and CD123^{dim}) from a control, SVR, and NR were isolated in an identical fashion and utilized in a quantitative PCR assay for TNFR1.

The primers utilized were obtained as follows. The NCBI database was first interrogated to find the sequence of the cytokine receptor or other receptors of interest and then entered into the Primer Express software program in order to generate a primer which was then optimized according to the parameters of the real time PCR (Table 1). The threshold cycle or $\mathbf{C_t}$ value, the lowest cycle in which fluorescence was detected during the exponential phase, was calculated where the exponential phase of the reaction began.

2.3. Standardization and Comparison of Samples. The C_t values were standardized using RNA isolated from cell lines known to express high levels of the target genes. In order to normalize across experiments, these values were compared to the same standard for all experiments, that is RNA from cell lines expressing high levels of TNFR or LTβR (PHA activated lymphocytes and THP-1, resp.). The resulting cycle threshold values were normalized to endogenous cyclophillin values and a calibrator value for each experiment. Raw cycle threshold values for the no template control within each experiment were significantly different than the experimental values for cyclophillin, CD3ε, NGFR, CD30, TNFR1, TNFR2,

Table 1: Real-Time PCR Primers.

Target	Primer Sequence
Cyclophillin	Fw 5′-TGCCATCGCCAAGGAGTAG-3′
	Rv 5′-TGCACAGACGGTCACTCAAA- 3′
TNFR1	Fw 5′-CGCTACCAACGGTGGAAGTC- 3′
	Rv 5′ -CAAGCTCCCCCTCTTTTTCAG-3′
LTβR	Fw 5′-CGGGCCCCTCTAAAGGATT-3′
	Rv 5′-GTGAAGTGTGGAACCCCAAAG-3′
TNFR2	Fw 5′-CAAGCCAGCTCCACAATGG-3′
	Rv 5′-TGACCGAAAGGCACATTCCT-3′
NGFR	Fw 5′-CCGTGGAGATGGGATGCTT-3′
	Rv 5′-TTTCCACGAACCCCAAACC-3′
CD30R	Fw 5′-GCTTTACTCTGGACCATAGGAAACA-3′
	Rv 5′-CTCCTTAGCGTGAAATGTGAAAAA-3′
IL2Rα	Fw 5′-CAGAAGTCATGAAGCCCAAGTG-3′
	Rv 5′-GGCAAGCACAACGGATGTCT-3′
CD27	Fw 5′-TCGGCACTGTAACTCTGGTCTTC-3′
	Rv 5′-CGACAGGCACACTCAGCATT-3′
FAS	Fw 5′-CTTTTCGTGAGCTCGTCTCTGA-3′
	Rv 5′-CTCCCCAGAAGCGTCTTTGA-3′
CD3	Fw 5′-CATCCCAAAGTATTCCATCTACTTTTC-3′
	Rv 5′-CCCAGTCCATCCCCAGAGA-3′

LTβR, FAS, IL-2Rα, and CD27, verifying the presence of cDNA for these receptors.

The comparison between control HCV(−) patients and SVR, NR, and Rel were expressed as fold elevation above control. The control population is defined as both HCV(−) patients with ALD or NAFLD ($n = 9$) and HCV(−) control subjects ($n = 10$).

2.4. Western Blot Analysis. Whole PBMC's from one SVR with a high level of TNFR1 mRNA by real time PCR were analyzed by Western blot in order to determine protein levels of TNFR1. Whole PBMC's were freeze-thawed twice, suspended in PBS, and assayed for protein, and 20 μg from one SVR and one control as well as 200 ng of recombinant human soluble TNF receptor 1 (R&D Systems, Minneapolis/St. Paul, MN) were prepared for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in the presence of 2-mercaptoethanol, resolved on a 10% acrylamide gel, and transferred to a nitrocellulose membrane. After blocking in 5% milk in TBS (0.1 mol/L NaCl and 0.4 mmol/L Tris, pH 7.4), containing 0.05% sodium azide, the blot was exposed to human soluble TNF receptor 1 polyclonal detection antibody (R&D Systems). Bound antibody was allowed to react with alkaline phosphatase-conjugated goat antimouse IgG (Sigma Chemical Co., St. Louis, MO) [15].

2.5. Flow Cytometry. At 44 weeks of therapy, whole PBMCs from a SVR and an NR were isolated from 40–60 cc whole blood by density gradient separation. In another experiment, PBMCs from an SVR at 48 weeks, NR at 24 weeks, and control patient were isolated as previously described for subset analysis. The PBMC's from all patients were suspended in staining buffer (0.5 L HBSS, 0.5 L PBS, 2% BSA, 0.02% sodium azide). Cells were labeled with the FITC-labeled anti-TNFR1 Ab (FAB225F, R&D Systems) or control FITC $IgG_1 6$ (555748l, BD Pharmingen). The samples designated for cell subset analysis were then stained with CD33 (555748l, BD Pharmingen), CD14 (555748l, BD Pharmingen), CD11 (555748l, BD Pharmingen), CD123 (555748l, BD Pharmingen), or relative isotype controls. After mAb staining and washing, all samples were fixed in PBS containing 1% paraformaldehyde at room temperature for 10 minutes and stored at 4°C until flow cytometric analysis as previously described in [16, 17]. Flow cytometric analysis was performed on an FACscan (Becton Dickinson) and used to determine the mean fluorescence intensity (MFI) of TNFR1 on all labeled cells. TNFR1 MFI ratios (TNFR1 MFI/isotype control MFI) were determined.

RNA from FAC sorted plamacytoid dendritic cells (PDCs) (CD11− and $CD123^{high}$) and myeloid dendritic cells (MDCs) (CD11+ and $CD123^{dim}$ from a control, SVRs and NR were utilized in a real time PCR assay for TNFR1.

2.6. Statistical Analysis. Descriptive data were reported as a mean for all standard deviation (SD) and median and range in Figures 1 and 2. All results were analyzed with an intention to treat analysis. Characteristics of treatment groups were compared using student's t-test. All results were analyzed using an exact Fisher test. A "P" value of less than .05 was considered to be significant.

3. Results

3.1. PBMC Tumor Necrosis Factor Receptor (TNFR) mRNA Levels from Responders to IFN Therapy Increased while Other TNFR Family Members mRNA Levels Remained Stable. RNA from PBMC of 23 HCV+ patients was isolated prior to the commencement of IFNα based therapy. Similarly, RNA from PBMC's of 10 HCV(−) controls and 11 patients with ALD or NAFLD were also isolated. During the course of 48 weeks of IFNα based therapy, 80 RNA samples were isolated from PBMC's from 13 HCV+ responder [(SVR and end of treatment responders (ETR)] patients and from 24 NR patients. Quantitative real time PCR levels of TNFR1, TNFR2, LTβR, FASR, CD30, CD27, and NGF, as well as CD3ε and IL-2Rα mRNA were assessed in available samples at 8, 12, 24, and/or 48 weeks of therapy (Table 2). In order to normalize across experiments, these values were normalized to the same standard for all experiments, that is, RNA from cell lines expressing high levels of TNFR (PHA-activated lymphocytes).

As noted, TNFR1 mRNA levels of PBMC from HCV+ responder patients were significantly higher than levels from control age- and sex- matched HCV(−) control patients during the course of the IFNα based therapy ($n = 26$, $P = .033$), while TNFR1 mRNA levels of PBMC's from HCV NR patients were not significantly different from controls ($n = 38$, $P = .20$). Similarly, during the course of IFNα based therapy, TNFR2 mRNA levels of PBMC from HCV+ responder patients were significantly higher than levels in

Table 2: TNFR1 Family Members' mRNA Levels.

	ControlHCV neg	Responders*		NR/Rel	
		Prior to Tx	On Tx	Prior to Tx	On Tx
TNFR1	0.83 ± 0.9 ($n = 10$) 1.2 ± 1.53 ($n = 11$)**	1.94 for all 2.43 ($n = 7$)	7.09 for all 8.82 ($n = 26$) ($P = .033$)**	2.83 for all 2.87 ($n = 14$)	2.31 for all 3.53 ($n = 38$) ($P = .20$)***
TNFR2	2.10 ± 2.93 ($n = 10$) 2.03 ± 1.34 ($n = 5$)**	1.36 for all 1.22 ($n = 7$)	5.93 ± 7.61 ($n = 22$) ($P = .045$)**	2.30 for all 2.67 ($n = 12$)	2.57 for all 4.60 ($n = 33$) ($P = .41$)**
LTβR	1.67 ± 1.01 ($n = 10$) 2.17 ± 2.66 ($n = 10$)**	1.21 for all 1.24 ($n = 7$)	1.49 ± 1.64 ($n = 22$) ($P = .75$)**	1.35 for all 1.35 ($n = 12$)	2.47 for all 5.41 ($n = 34$) ($P = .66$)**
FASR	0.38 for all 0.54 ($n = 3$)	0.001 ($n = 1$)	0.14 for all 0.40 ($n = 18$) ($P = .35$)**	0.01 for all 0.01 ($n = 6$)	0.03 for all 0.07 ($n = 21$) ($P = .003$)**
CD30	0.37 for all 0.41 ($n = 6$)	0.175 for all 0.06 ($n = 2$)	0.61 for all 1.22 ($n = 21$) ($P = .65$)**	0.06 for all 0.07 ($n = 5$)	0.61 for all 1.51 ($n = 20$) ($P = .71$)**
CD27	0.86 for all 0.49 ($n = 6$)	0.77 for all 0.37 ($n = 2$)	1.08 for all 0.65 ($n = 21$) ($P = .45$)**	1.10 for all 0.62 ($n = 5$)	0.71 for all 0.62 ($n = 20$) ($P = .58$)**
NGF	0.75 for all 1.22 ($n = 6$)	0.085 for all 0.007 ($n = 2$)	1.85 for all 4.80 ($n = 22$) ($P = .59$)**	0.11 for all 0.19 ($n = 6$)	1.37 for all 2.86 ($n = 18$) ($P = .62$)**
IL-2R for all	0.22 for all 0.39 ($n = 6$)	0.175 for all 0.18 ($n = 2$)	0.45 for all 0.63 ($n = 21$) ($P = .41$)**	0.10 for all 0.22 ($n = 6$)	0.05 for all 0.10 ($n = 19$) ($P = .09$)**
CD3	1.43 for all 0.71 ($n = 3$)	1.3 ($n = 1$)	4.71 for all 12.62 ($n = 17$) ($P = .67$)**	1.67 for all 1.07 ($n = 5$)	6.93 for all 21.06 ($n = 18$) ($P = .66$)**

*Include End of treatment responders (ETR) and SVR.
**Mean value for control liver disease patients, HCV negative.
***P-value compared to control (HCV negative, age and sexed matched).

the control age- and sex- matched HCV(−) patients ($n = 22$, $P = .045$) while no difference was noted relative to levels in HCV NR and controls ($n = 33$, $P = .41$). No other significant increases were observed in the other TNFR family members or T-cell-associated mRNA (CD3ε or IL-2Rα) levels of PBMC isolated from responders or NR (Table 2). In summary, PBMC's mRNA levels of TNFR1 and TNFR2 from responders to IFNα based therapy were statistically different than levels in control HCV(−) age- and sex- matched patients.

3.2. TNFR1 and TNFR2 mRNA Levels from HCV+ Patients Prior to Treatment and Control Patients Were Similar. In light of differences between levels of TNFR1 and TNFR2 mRNA in PBMC of responders and nonresponders during the therapy, further analysis of the TNFR1 and TNFR2 levels was performed. RNA was isolated from PBMCs of 23 HCV+ untreated patients, 10 control HCV(−) patients, and 11 patients with ALD or NAFLD. TNFR1 and TNFR2 mRNA levels were similar in both HCV infected and control groups [1.02 ± 1.25 ($n = 21$) versus 2.53 for all 2.71 ($n = 23$) and 1.95 for all 2.42 ($n = 15$) versus 2.07 for all 2.45 ($n = 18$)] (Figure 1, panels A and B). Quantitative real time PCR revealed no difference between initial levels of TNFR1 mRNA in the HCV+ genotype 1 patients and control non-HCV+ patients ($P = .08$) nor between HCV+ genotype 1 patients and patients with non-HCV liver disease ($P = .17$). Similar results between initial TNFR1 RNA levels in control (HCV negative and non-HCV liver disease) and initial levels in HCV+ patients were noted [median 0.59 (Range: 0.02–5.42)] versus [median 1.52 (Range: 0.06–11.65)] (Figure 1, panel A). TNFR2 mRNA levels were not significantly different between HCV+ patients prior to therapy and either HCV(−) control patients or HCV(−) patients with liver disease ($P = .88$ and $.95$), (Figure 1, panel B).

3.3. Peak TNFR1 mRNA Levels Were Significantly Different between SVR and Nonresponders. In order to determine whether the elevation of TNFR1 or TNFR2 mRNA was above control levels at any point during IFNα based therapy (4, 8, 12, 24 and 48 weeks), 12 genotype 1 HCV+ SVR and 25 genotype 1 HCV+ patients NR/Rel were evaluated. TNFR1 and TNFR2 mRNA elevations were significantly more frequent in SVR versus NR/Rel.

The peak TNFR1 mRNA levels in PBMC of genotype 1 HCV+ patients at 4, 8, 12, 24, or 48 weeks were greater than 2 SD (2.63 fold elevation) above mean TNFR1 mRNA levels from HCV(−) controls in 7/12 (58%) sustained virological responders (SVR), but in only 5/21 (28%) genotype 1 NR and 2/4 (50%) genotype 1 Rel. Furthermore, the peak TNFR1 mRNA levels from responders were significantly higher than the peak TNFR1 mRNA levels in NR and Rel ($P = .0023$)

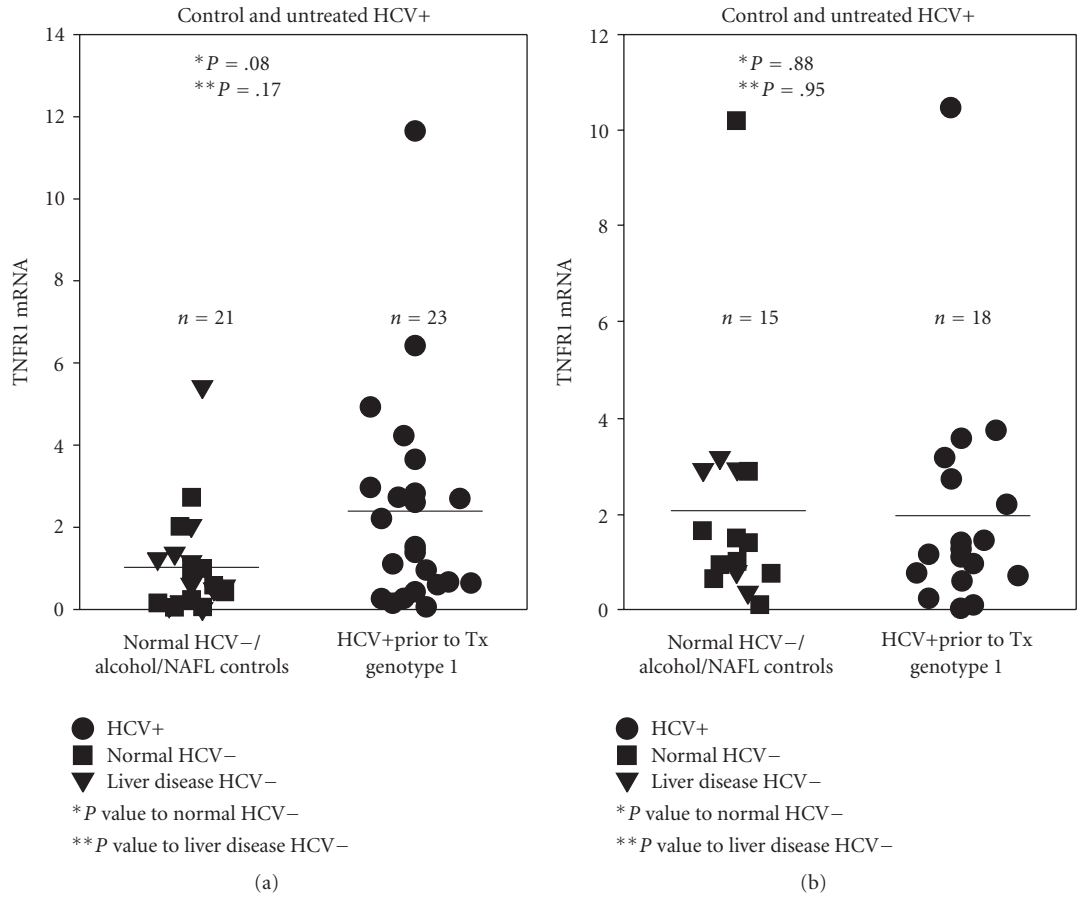

Figure 1: TNFR1 and TNFR2 mRNA levels were not different between HCV(−)/ALD/NAFLD control patients and genotype 1 HCV+ patients prior to treatment. TNFR1 mRNA levels of PBMC's isolated from 10 HCV(−) controls, 11 ALD/NAFLD controls, and 23 HCV+ patients prior to therapy were ascertained by real time PCR (Panel A). TNFR2 mRNA levels of PBMC's isolated from 10 HCV(−) controls, 5 ALD/NAFLD controls, and 18 HCV+ patients prior to therapy were ascertained by real time PCR (Panel B). Statistical differences were assessed by *T*- test.

[SVR: Median 7.37 (Range: 0.29–35.92) versus NR/Rel: Median 1.17 (Range: 0.08–15.91)] (Figure 2(a)).

The peak TNFR2 mRNA levels expressed in PBMC from genotype 1 HCV+ patients at 4, 8, 12, 24 or 48 weeks were greater than 2 SD (8.6 fold elevation) above mean control TNFR2 mRNA levels from HCV(−) patients in 3/12 (25%) SVR versus 3/20 (15%) genotype 1 NR and 0/4 (0%) genotype 1 Rel. The peak TNFR2 mRNA levels from SVR were not significantly higher than the peak TNFR2 mRNA levels from NR and Rel ($P = .085$) (Figure 2(b)). Similar results of peak TNFR levels in responders and nonresponders were noted [median/range 6.1 (0.71–32.01) versus 2.98 (0.01–18.57) in Figure 2(b)]. Therefore, unlike peak PBMC TNFR1 mRNA levels, peak PBMC TNFR2 mRNA levels during IFN for all therapy were not significantly different between SVR and nonresponders.

3.4. TNFR1 Expression by HCV+ SVR Patient's PBMCs Was Demonstrated by Western Blot Analysis. In order to assess whether TNFR1 mRNA levels directly correlated with differential expression of TNFR1 protein in a SVR or control PBMCs, whole PBMC from a HCV+ SVR and a control HCV(−) patient were lysed, assayed for protein content, and 20 μg of protein per sample was loaded on a SDS PAGE gel. Detection of the protein of −55 kDa molecular weight by anti-TNFR1 was observed in the PBMC of an HCV+ responder patient but was not observed in the HCV(−) control PBMC lysate (Figure 3).

3.5. Increased TNFR1 Membrane Expression by Specific Peripheral Blood Monocyte Subsets from SVR but Not NR Was Observed. The surface expression of TNFR1 by PBMC in HCV+ patients undergoing IFN/R has not been reported. However, soluble TNFR1 has been described to be elevated in patients with chronic HCV [6]. Because of the differences noted in the TNFR1 mRNA levels between NR and the SVR, flow cytometric analysis of TNFR1 expression by PBMC from a SVR and a NR was assessed. Whole PBMCs from NR and SVR were isolated and stained with an FITC-conjugated anti-TNFR1 Ab as described in the methods. The PBMC of the HCV+ SVR patient displayed 52% TNFR1 positive PBMC with mean fluorescence intensity (MFI) of 80. The non-responding patient displayed 0.0% TNFR1 positive PBMC's, with MFI of 0. As demonstrated by the flow

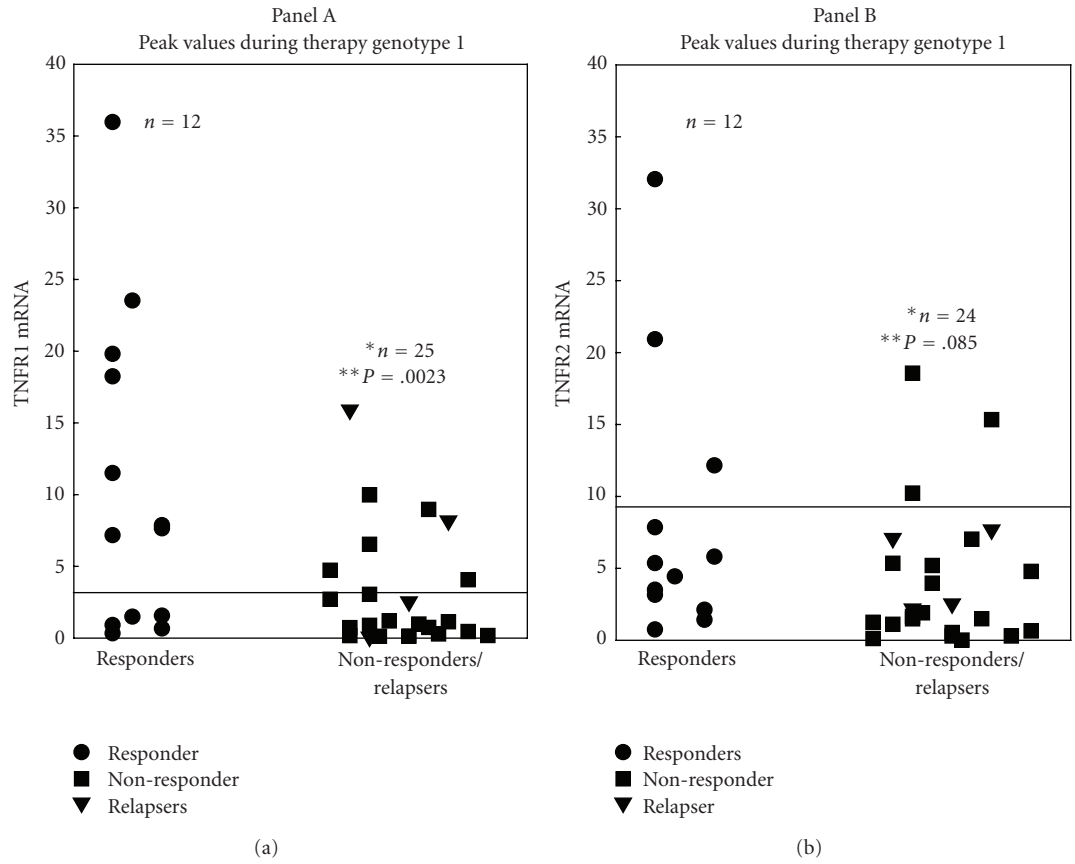

Figure 2: Peak TNFR1 mRNA values were higher in responder patients than nonresponder patients. TNFR1 mRNA levels of genotype 1 HCV+ patients PBMC's were ascertained by real time PCR. Twelve responders, 25 NR/Rel and 12 responders, 24 NR/Rel were assessed for TNFR1 and TNFR2 mRNA levels, respectively (Panel A and B). Line indicates 2 SD above mean control levels. Levels did not vary between sample triplicates. Statistical differences were assessed by T test.

cytometric figure, the increase of TNFR1 levels was noted in cells with light scatter characteristics of peripheral blood monocytes or dendritic cells (Figure 4).

In order to further delineate which PBMC subset had elevated levels of TNFR1 expression, TNFR1 MFI ratios (TNFR1 MFI/isotype control MFI) were compared between SVR, NR/Rel, and controls. HCV+ patients prior to therapy and uninfected controls were noted to have similar TNFR1 MFI ratios in both the CD33+ (1.2 ± 0.01 ($n = 2$) versus 1.56 ± 0.37 ($n = 2$), resp. $P = .19$) and CD123+ PBMC (1.14 ± 0.56 ($n = 2$) versus 1.40 ± 0.09, ($n = 2$) resp., $P = .57$). No difference was observed between the TNFR1 MFI ratios from both CD33+ and CD123+ PBMC of the uninfected controls and the NR ($P = .4$ and $P = 1.0$, resp.).

In order to further assess the specific cell subset affected by IFNα, 24 PBMC samples were stained with CD123, CD33, and TNFR-1 (16 SVR and 8 NR/Rel). Sixteen samples were obtained from 5 SVR from weeks 62–48, and 8 samples were taken from NR/Rel from weeks 5–81. A trend toward a lower TNFR1 expression in CD123+ dendritic cells subset was noted in the NR/Rel group (1.25 ± 0.45 versus 1.59 ± 0.42, $P = .052$). Of interest, a significant difference was noted between the peak value of TNFR MFI ratio (highest TNFR1 MFI ratio observed during therapy) in CD123+ PBMC isolated from SVR and NR/Rel (Table 3).

Further cell subset identification was performed utilizing an analysis by flow sort using three cell markers to identify the two cell subsets of dendritic cells: myeloid (MDC) and plasmacytoid dendritic cells (PDCs). In the first panel, the control patient without chronic liver disease had virtually no TNFR1 expression, in all 3 subsets, CD14 + CD123+, MDC (CD11+ and $CD123^{dim}$), and PDC (CD11− and $CD123^{high}$) (Figure 5(a)). However, in the second panel, after 48 weeks of Ifn/R therapy, expression of TNFR1 in MDC and PDC in the SVR was higher compared to the control (Figure 5(b)) In the non responder group the expression of TNFR1 was similar to controls, low in all three subsets (Figure 5(c)). Hence, upregulation of TNFR1 membrane expression in the dendritic cell subset isolated from the SVR was noted.

3.6. TNFR1 mRNA Levels Increased during IFNα Based Therapy and Was Isolated to the Plasmacytoid Dendritic Cells. In order to determine when TNFR1 mRNA levels increased during IFN for all based therapy, 13 genotype 1 SVR and 12 genotype 1 NR or Rel were evaluated. In twenty-five genotype 1 HCV+ patients enrolled in IFN/R therapy with

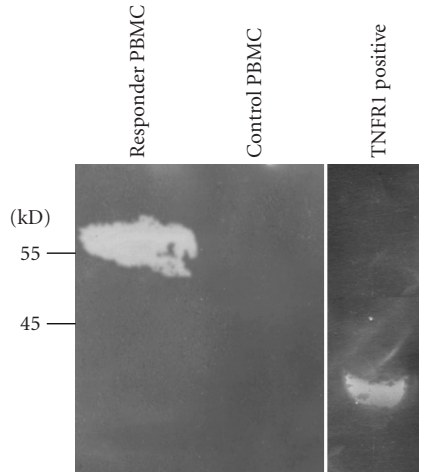

Figure 3: TNFR1 protein expression was observed in PBMC's from HCV+ responder patient. Whole PBMC's from a responding HCV+ patient and a control HCV(−) patient were lysed and assayed for protein content. Protein (20 μg) was loaded for responder (lane 1) and HCV(−) patient (lane 2) and 0.2 μg TNFR1 chimeric protein positive control (lane 3) was loaded and subjected to SDS-PAGE.

PBMC mRNA samples collected during the 48 weeks of therapy, patients with SVR ($n = 10$) were more likely to have increases in TNFR1 mRNA levels above mean control levels than patients with NR/Rel ($n = 3$) versus 3/12 (25%) NR/Rel. Importantly, the peak levels in the NR/Rel were lower than in the SVR (Tables 4(a) and 4(b)). Of interest, 4/7 (56%) patients with elevated TNFR1 mRNA values during therapy remained above mean control levels weeks after interferon therapy discontinuation.

RNA from FAC sorted plasmacytoid dendritic cells (PDCs) (CD11− and $CD123^{high}$) and myeloid dendritic cells (MDCs) (CD11+ and $CD123^{dim}$) from a control, SVR, and NR at 28 weeks of therapy were utilized in a real time PCR assay for TNFR1. TNFR1 mRNA levels in PDC isolated from a responder were 6.7 fold higher than the level of the control PDC. No difference in TNFR1 mRNA levels was noted in the control compared to responders MDC, non responders MDC, or non responders PDC. TNFR 1 RNA level is higher in the PDC isolated from patients with SVR than patients with NR at 28 weeks (Figure 6).

4. Discussion

This study is the first paper detailing the levels of TNFR family member expression in PBMC samples from responders and nonresponders to IFN therapy for chronic HCV over a 12-month period. Both TNFR1 and TNFR2 mRNA levels in PBMC isolated during therapy were higher in responders than control HCV(−) patients. Notably, the peak values of PBMC TNFR1 mRNA during therapy were higher in sustained virological responders than nonresponders or relapsers. Furthermore, PBMC expression of other members of the TNFR family remained stable during the course of the therapy. Importantly, the TNFR1 mRNA upregulation occurred in a subset of PBMC, with cell surface markers consistent with the dendritic cells.

Though other investigators have noted an increase in soluble TNFR1 (sTNFR1) and soluble TNFR2 (sTNFR2) in the serum of chronic HCV patients (18–21), this study is the first to note that the level of PBMC TNFR mRNA rises over the course of IFN based therapy in responders. Furthermore, others have noted that sTNFR2 appears to be significantly correlated with the severity of the disease and

Table 3

Peak Values	SVR	NR/Rel	P value
TNFR1 MFI Ratio	$N = 5$	$N = 6$	
CD33+	2.2 ± 0.56	1.54 ± 0.57	.06
CD123+	2.06 ± 0.59	1.21 ± 0.43	.01

Table 4: (a) TNFR1 mRNA Levels in genotype 1 SVR during IFN based therapy. (b) TNFR1 levels in genotype 1 non-responders/relapsers during IFN-based therapy.

(a)

	week 0	week 4–12	14–24 wks	25–48 wks
Patient 1	0.96	7.83		
Patient 2	1.39	7.13	1.97	0.71
Patient 3	4.23		7.61	3.86
Patient 4			8.44	23.48
Patient 5	0.15		0.63	
Patient 6	6.42		19.77	11.73
Patient 7			0.87	0.82
Patient 8			0.29	1.45
Patient 9			0.15	11.46
Patient 10			1.24	1.52
Patient 11	0.43		35.93	8.04
Patient 12	0.06			18.20
Patient 13		0.29	0.22	

(b)

	week 0	week 4–12	14–24	25–48
Patient 1	1.53	4.73		
Patient 2	0.67		1.14	
Patient 3	2.73		0.17	
Patient 4	2.61			0.98
Patient 5	2.97		6.54	
Patient 6	0.64	4.07		0.16
Patient 7			1.25	2.71
Patient 8	2.83		1.20	0.34
Patient 9	0.26		0.10	0.11
Patient 10			0.73	0.15
Patient 11			0.08	0.05
Patient 12			0.46	0.14

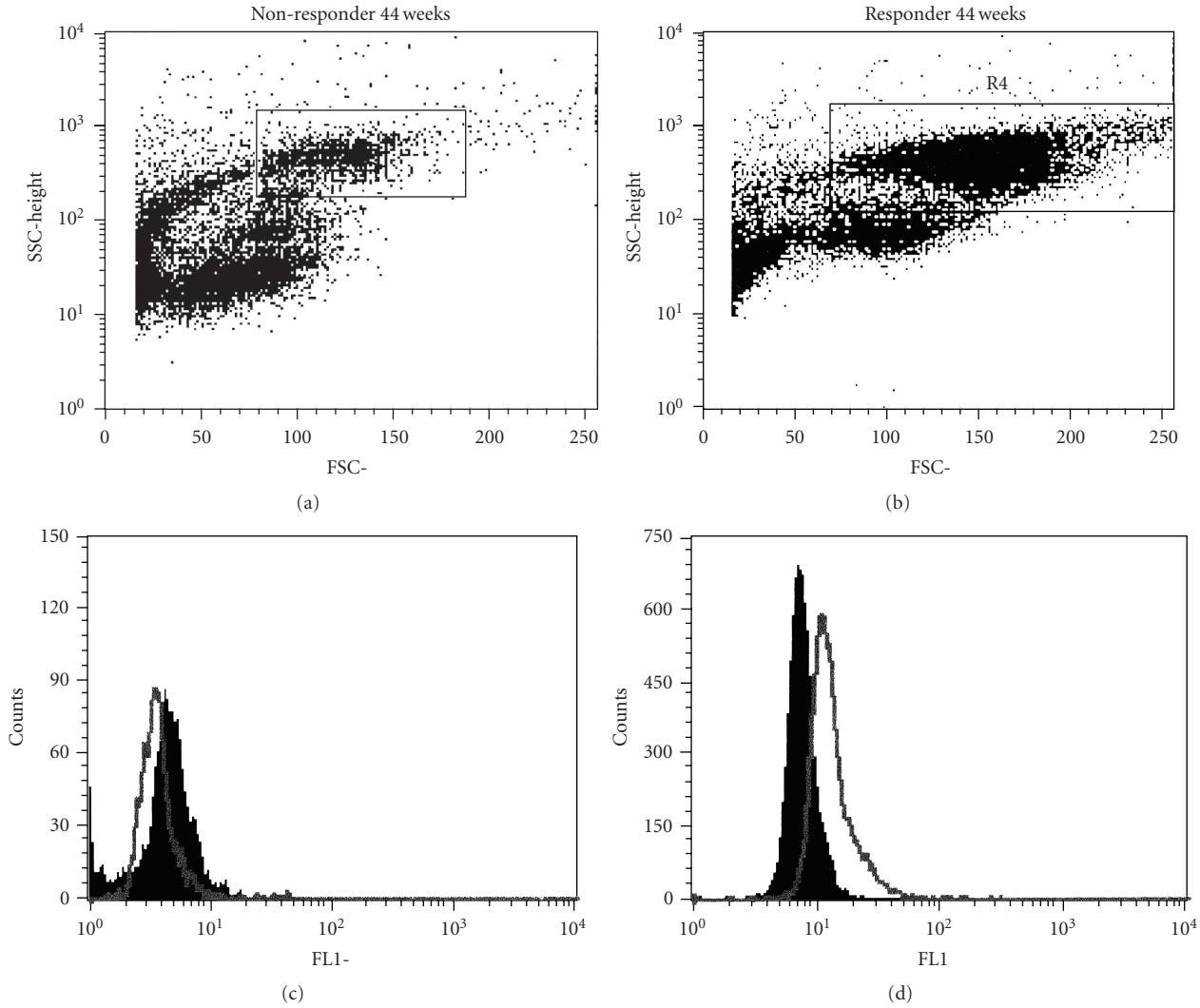

Figure 4: Flow cytometry revealed an increased frequency of PBMC's that expressed TNFR1 among responders. Whole PBMCs were stained with FITC TNFR1 as described in the methods. Dot plots are noted on (a) and (b) with regions analyzed on each dot plot. (c) and (d) demonstrate the responder and the nonresponder histograms of TNFR1 in the specific region outlined in (a) and (b).

fibrosis [5]. As noted in our previous publications, 50%–60% of patients treated at the Dallas VA have stage 3 fibrosis [18], and in part enhanced TNFR1 expression at baseline may be associated with fibrosis scores. However, there have been no significant differences in fibrosis between responders and nonresponders to therapy in our patient population [18].

Though the upregulation of TNFR1 may be associated with prolonged exposure to IFN, patients with multiple sclerosis (MS) treated with long term type 1 IFN (interferon beta) had stable PBMC TNFR 1 mRNA levels (data not shown). Other mechanisms besides prolonged exposure to type 1 IFN, involved in TNFR1 upregulation may include clearance of HCV virus from specific cell types, allowing further signaling from TNFR1 to NF6B, inducing TNFR1 upregulation [4]. Other mechanisms may relate to improved antigen presentation by APC's in specific patient populations after IFN exposure. Finally, specific patient populations may have different genetic polymorphisms of TNFR1, which allow for enhanced viral clearance and upregulation of the receptor's mRNA, accounting for differences in response to IFN based therapy.

Though there are limitations in the study in that less than half of the patients with SVR had both initial and long term followup, there is a suggestion that TNFR1 RNA levels may increase in patients that respond and that this increase appears be related to an upregulation of TNFR1 on a dendritic cell subset. Though this represents only a portion of the patients, the authors are suggesting that the findings in this study bear reporting.

The finding that dendritic cell subset appears to be the site of TNFR1 upregulation may provide insight into the cause, these cells have been implicated as the cell among PBMC that contains HCV negative RNA strand. Though only a portion of the patients had extensive flow cytometric analysis, the results suggest that the dendritic cell is at least one of the cells, where upregulation of TNFR1 occurs. While

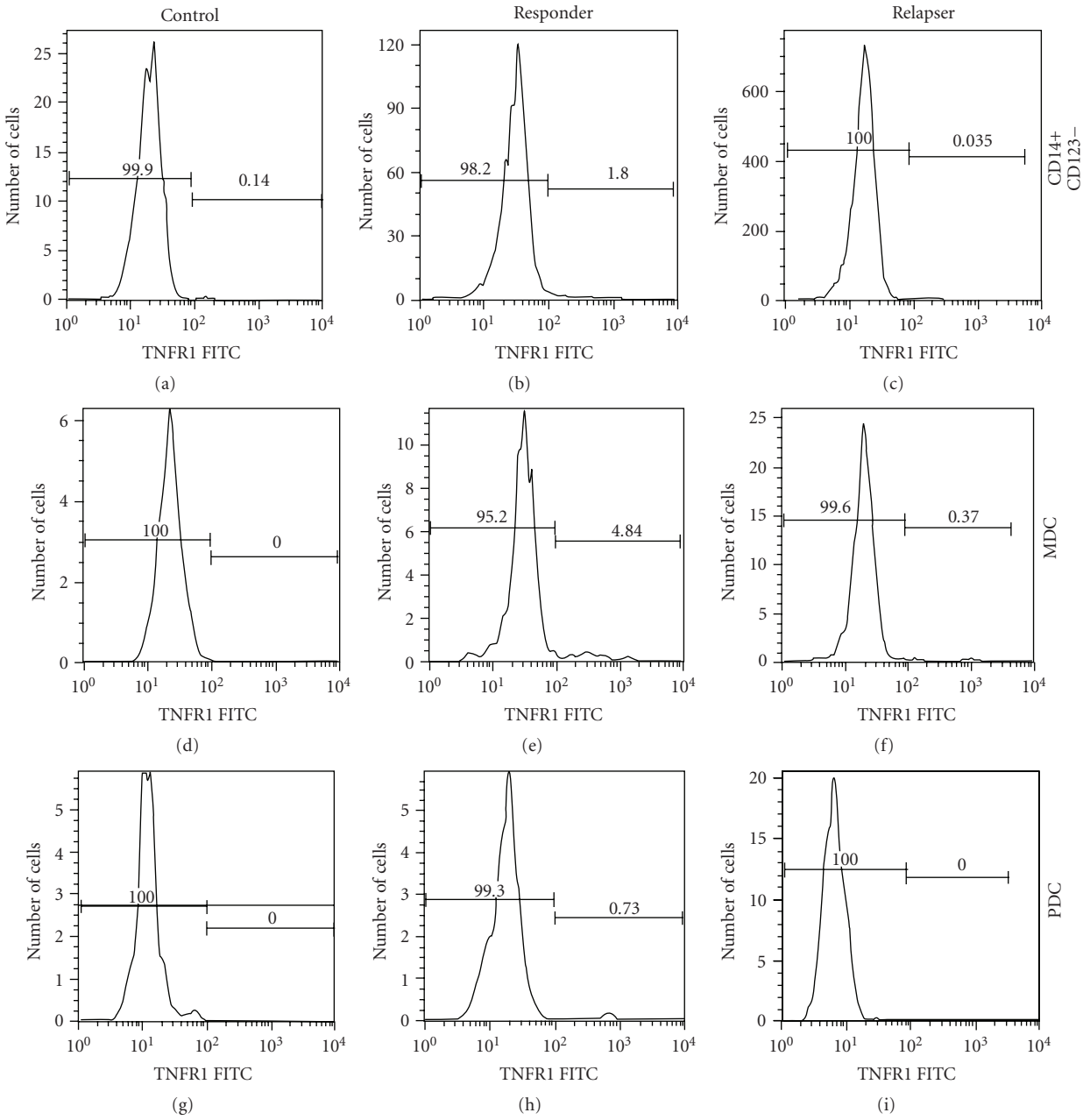

Figure 5: TNFR1 membrane expression is higher in dendritic cell subset in SVR compared to NR. Whole PBMCs from control HCV−, SVR and NR were stained with PE-labeled CD123, FITC-labeled CD11, Texas Red-labeled CD14, and/or relative isotype controls as previously described in the methods. CD11+, CD123^{dim} , CD11−, CD123^{high}, and CD14+, CD11+ cell subsets were identified by 2-color analysis. Significant differences were noted in the expression of TNFR1 in the histogram from selected cell subset from a control HCV− (column a), SVR (column b), and NR (column c).

recent reports suggest that PBMC's from up to 67% of SVR patients still express residual HCV RNA, these levels are likely greatly reduced from pretherapy levels and relief of HCV-mediated expression of TNFR family signaling may explain rebound enhanced expression of TNFR1. Furthermore, these APC's have also been implicated as being critical for viral clearance. Our study supports that within these cells, a cell surface receptor, TNFR1, is disparately upregulated in patients responding to IFN based therapy.

Initially, TNFR1 expression was noted to be upregulated in CD123+ dendritic cells in patients with sustained virological response. The data supports that at least in some patients the increase in TNFR1 mRNA is predominantly found in the plasmacytoid dendritic cells, an avid antigen presenting cell. Importantly, upregulation of TNFR1 surface expression was noted in one SVR patient's myeloid dendritic cell, suggesting that both types of dendritic cells may be important in HCV viral clearance.

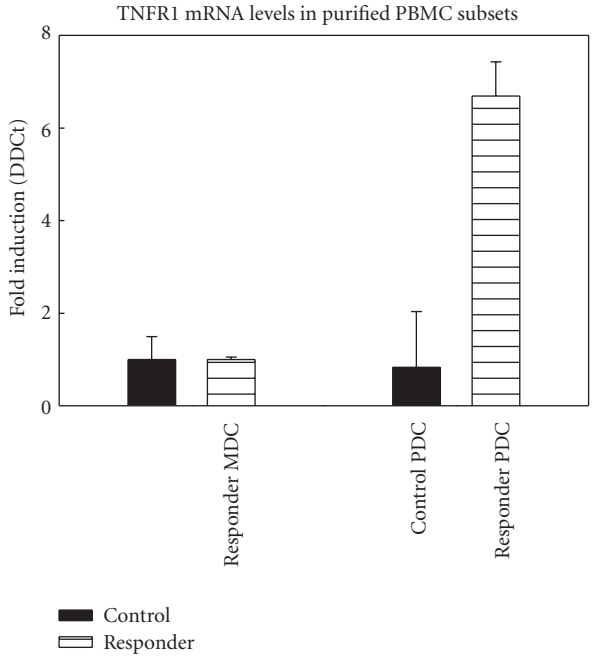

Figure 6: The increase in TNFR1 mRNA levels was isolated to the plasmacytoid dendritic cells. RNA from FAC sorted plamacytoid dendritic cells (PDC) (CD11− and CD123^{high}) and myeloid dendritic cells (MDC) (CD11+ and CD123^{dim}) from a control, SVR, and NR were used in a real time PCR assay for TNFR1. Statistical differences were assessed by *T* test.

In summary, TNFR1 mRNA and protein levels are upregulated in dendritic cells. While the mechanism of this upregulation has yet to be elucidated, this cell subset may be involved in antigen presentation of viral proteins, and TNFR1 levels may correlate with recovery of effective APC function.

Acknowledgments

Bonnie Whittington and George Lane are acknowledged for their excellent technical assistance.

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Hindawi Publishing Corporation
Hepatitis Research and Treatment
Volume 2010, Article ID 495928, 5 pages
doi:10.1155/2010/495928

Clinical Study

Optimal Erythrocyte Ribavirin Level to Reduce the Risk of Anemia and Obtain an Early Virological Response in Patients with Chronic Hepatitis C Caused by Genotype 1b Infection

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Received 13 May 2010; Revised 28 July 2010; Accepted 4 August 2010

Academic Editor: Tatehiro Kagawa

Aims. To determine whether the erythrocyte phosphorylated ribavirin (RBV) level might be a useful index of EVR and risk of anemia and to determine the optimal dose of RBV in 24 patients with hepatitis C with pegylated interferon and RBV. *Methodology*. The RBV level was measured by a high-performance liquid chromatography. *Results and Conclusion*. In patients aged 50 years or over, a negative correlation ($r = -0.548$, $P < .05$) was observed between the RBV level at week 2 and rate of Hb reduction (ΔHb) at week 4. The ΔHb at week 4 was significantly greater in patients with RBV levels of $\geq 800\,\mu$M ($-25.5 \pm 10.1\%$) than in patients with RBV levels $<800\,\mu$M ($-15.6 \pm 7.7\%$). None of the patients with RBV levels $<600\,\mu$M at week 2 achieved EVR and SVR. Thus the optimal levels of erythrocyte phosphorylated RBV at week 2 of therapy in order to achieve EVR without anemia seemed to be 600–800 μM.

1. Introduction

The combination therapy with pegylated interferon (PEG-IFN) and ribavirin (RBV) has come to be established as the standard treatment for chronic hepatitis C. A sustained virological response (SVR) has been reported with this treatment in 30%–50% of patients with HCV genotype 1b infection, which accounts for 70% of all Japanese patients with chronic hepatitis C [1]. However, the treatment often needs to be discontinued, or the dose of RBV changed, in these patients due to the development of hemolytic anemia. On the other hand, continuous treatment is important to obtain SVR with the treatment [2].

RBV is incorporated into the cells via the equilibrative nucleoside transporter (ENT) and converted to phosphates within the cells. RBV monophosphate (RMP) and RBV triphosphate (RTP) are considered to have antiviral activity [3, 4]. In nucleated cells, the phosphorylated RBV is subsequently dephosphorylated by the dephosphorylating enzyme, and RBV is eliminated from the cells via the ENT. However, in akaryocytes such as erythrocytes, which lack the dephosphorylating enzyme, accumulation of phosphorylated RBV occurs, which diminishes the cellular ATP and alters the cellular characteristics; these changes in the characteristics of the erythrocytes activate the cell-elimination activity of the reticuloendothelial system, resulting in hemolysis [5, 6].

In this study, with the objective of reducing the adverse effects and improving the treatment completion rate in patients receiving combined PEG-IFN and RBV therapy, we attempted to evaluate whether the erythrocyte phosphorylated RBV level might be useful as an index for the rate

of Early Virological Response (EVR) and SVR, the risk of anemia.

2. Materials and Methods

2.1. Subjects. Among the patients with chronic hepatitis C caused by genotype 1b infection in whom combined PEG-IFNα2b and RBV therapy was started, 24 patients who provided written informed consent for participation in this study were enrolled. The dosage regimen for the combined PEG-IFN and RBV therapy was determined in accordance with the standard dosing recommended for Japanese HCV patients. RBV was started at the initial dose of 600 mg/day in patients with a body weight of ≤60 kg, 800 mg/day in those with a body weight of >60 kg and ≤80 kg, and 1,000 mg/day in those with a body weight of >80 kg. In patients with no cardiovascular disease, the RBV dosage of 600 mg/day was decreased to 400 mg/day, and 800 mg or 1000 mg/day was reduced to 600 mg/day, if hemoglobin (Hb) level decreased to less than 10 g/dL; permanently discontinued the drug if Hb decreased to less than 8.5 g/dL. In those with history of stable cardiovascular disease, the dosage of RBV was decreased to 400 mg or 600 mg if Hb decreased by 2 g/dL or more during any 4-week period; permanently discontinued the drug if Hb was less than 12 g/dL after 4weeks of a reduced RBV dosage. The patients were followed up until 48 weeks after the start of the combined IFN and RBV therapy, and Hb level and HCV RNA level were examined at week 12 after the start of treatment.

Serum HCV RNA negativity until 12 week of the therapy was defined as EVR. Additionally, serum HCV RNA negativity until 24 weeks after the therapy was completed and was defined as SVR. This study was performed with the approval of the hospital research committee, in compliance with the ethical principles laid out in the Declaration of Helsinki.

2.2. Measurement of the Erythrocyte Level of Phosphorylated RBV. Ten-mL samples of venous blood were obtained at 2, 4, and 8 weeks after the start of the therapy, and the erythrocyte level of phosphorylated RBV was measured by the HPLC method described by Homma et al. [7]. In this method, all phosphorylated RBV (RMP, RDP, and RTP) is converted back to RBV by treatment with an erythrocyte dephosphorylating enzyme, and the erythrocyte level of phosphorylated RBV is calculated as the difference in the RBV levels measured before and after the enzyme treatment.

2.3. Statistical Analysis. The changes in the Hb level were analyzed by repeated-measures ANOVA and Dunnett's test. The relationships of the RBV level to the risk of anemia and the drug efficacy were examined by Student's t-test, Pearson's correlation coefficient, χ^2-test, and Fisher's exact probability test. $P < .05$ was regarded as denoting clinical significance.

3. Results

3.1. Patient Characteristics and Therapeutic Course. The subjects comprised 24 patients, and their demographic characteristics are indicated in Table 1. The combined PEG-IFN and RBV therapy needed to be discontinued in 3 of the 24 patients (12.5%), and the RBV dose needed to be reduced in 7 of the patients (29.2%) due to the development of anemia (Hb ≤ 10 g/dL). One patient was discontinued the therapy due to adverse reaction except anemia. However, the conditions of RBV administration for the initial 4 weeks were not changed. None of those in whom this therapy was discontinued achieved EVR. There were no significant difference in the EVR rates between the subjects in whom the RBV dose was reduced (72.7%) and those in whom the therapy continued at the initial dose (64.0%). The dosage of PEG-IFN was 1.5 ± 0.2 μg/kg in accordance with a standard regimen, and the conditions of IFN administration have not changed for 48 weeks after the combination therapy was started in the subjects except for 4 patients who discontinued the therapy.

Table 1: Baseline characteristics of patients.

Characteristics	($n = 24$)
Age (years)	59.9 ± 10.3
Sex (M/F)	12/12
Past treatment (Yes/No)	15/9
Body weight (kg)	57.7 ± 9.1
Ribavirin (mg/kg/day)	11.7 ± 1.5
Hemoglobin (g/dL)	13.6 ± 1.3
HCV RNA (KIU/mL)	
<100	1
100~500	3
500~850	4
850 ≦	16

Data are expressed as mean ± S.D. or number of patients
HCV: Hepatitis C virus.

3.2. Changes in the Hb Level. The Hb levels (11.0 ± 1.3 g/dL) were significantly lower at week 4 of therapy as compared with 13.6 ± 1.3 g/dL at the start. In addition, the rate of Hb reduction [(Hb level-Hb level before administration)/Hb level before administration] at week 4 of therapy was −12.4% in those aged less than 50 years, whereas it was −21.0% in those aged 50 years or over ($P < .05$).

3.3. Changes in the Erythrocyte Phosphorylated RBV Level. The phosphorylated RBV and nonphosphorylated RBV levels in the erythrocytes were 749.3 ± 244.3 and 6.9 ± 3.6 μM at week 2, 1039.9 ± 239.6 and 8.4 ± 7.0 μM at week 4, and 907.9 ± 292.1 and 8.9 ± 8.0 μM at week 8 of therapy, respectively; thus, about 99% of the RBV in the erythrocytes was phosphorylated (Figure 1).

3.4. Relationship between the Erythrocyte Phosphorylated RBV Level at Week 2 and the Frequency of Anemia. The relationship between the RBV level at week 2 and the rate of reduction of the Hb level was examined in the 19 patients aged 50 years or over. There was a negative correlation ($r = -0.548$, $P < .05$) between the RBV level at week 2 and the rate

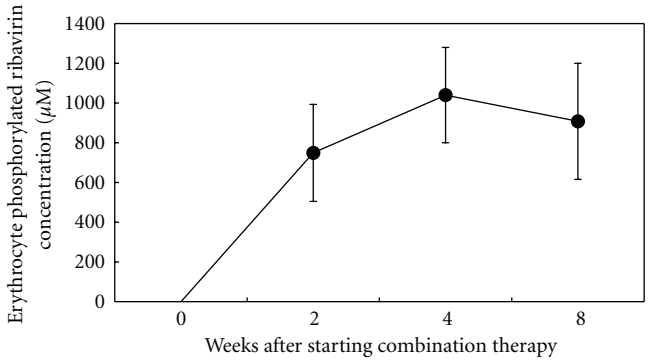

Figure 1: Time course of erythrocyte phosphorylated ribavirin concentration after starting PEG-IFNα-2b and ribavirin combination therapy.

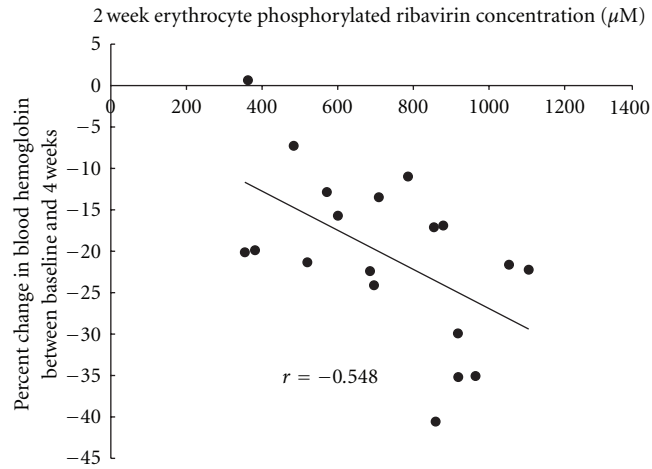

Figure 2: Correlation between 4-week hemoglobin reduction rate from the baseline and 2-week erythrocyte phosphorylated ribavirin concentration in patients aged 50 and over. $r = -0.548$ ($P < .05$).

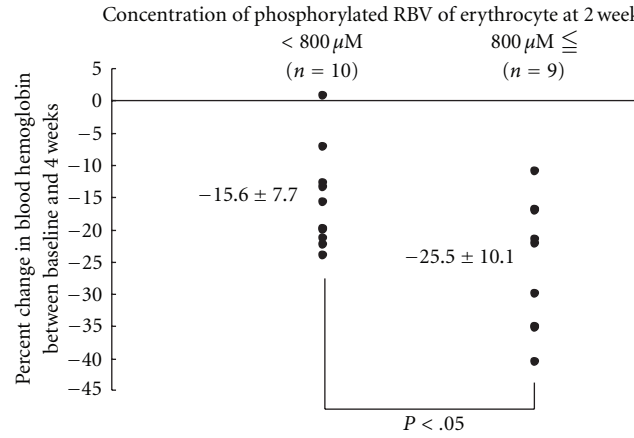

Figure 3: Comparison of 4-week hemoglobin reduction rate from the baseline of the patients with 2-week erythrocyte phosphorylated ribavirin concentration(<800 μM) to 4-week hemoglobin reduction rate from the baseline of the patients with 2-week erythrocyte phosphorylated ribavirin concentration(800 μM ≦).

of Hb reduction (ΔHb) at week 4 in the subjects in whom the dose of RBV did not reduce or the combination therapy did not discontinue until week 4 (Figure 2). As shown in Figure 3, the ΔHg at week 4 was significantly higher ($P < .05$) in those with RBV level of ≥800 μM ($-25.5 \pm 10.1\%$) than in those with the level of <800 μM ($-15.6 \pm 7.7\%$).

3.5. Relationship between the Erythrocyte Phosphorylated RBV Level at Week 2 and the EVR. The relationship between the phosphorylated RBV level at week 2 and the EVR was evaluated in 20 of the 24 patients (Four cases were excluded because of the discontinuation of the therapy). The mean RBV level at week 2 was significantly lower ($P < .05$) in the non-EVR patients ($634.6 \pm 236.6\,\mu$M) than the EVR patients ($889.7 \pm 210.6\,\mu$M).

As shown in Table 2, 3 cases with the phosphorylated RBV level in erythrocytes ≥800 μM discontinued the combination therapy prematurely due to anemia, whereas none of 14 cases with a levels <800 μM discontinued prematurely.

There were no EVR or SVR cases (0 of 7 cases) in patients with erythrocyte phosphorylated RBV levels <600 μM at week 2, whereas, in those with levels ≥600 μM, 11 of 17 cases (64.7%) had EVR and 6 of 17 cases (35.3%) had SVR ($P < .05$).

Five of 7 cases (71.4%) with erythrocyte phosphorylated RBV level at week 2 of 600–800 μM achieved EVR and 3 cases (42.9%) achieved SVR without development of marked anemia. None of those patients discontinued RBV due to development of anemia.

4. Discussion

In this study, combined PEG-IFN and RBV therapy needed to be discontinued, or the RBV dose needed to be reduced, in about 40% of the study subjects due to the development of hemolytic anemia (Hb ≤ 10 g/dL). None of the patients in whom the combined PEG-IFN and RBV therapy was discontinued by Week 12 showed SVR. However, no difference in the rate of SVR was noted between the subjects in whom the RBV dose was reduced and those in whom the treatment could be continued at the initial dose. This suggests that continuation of the combination therapy was the most important factor for achieving the desired clinical outcome. Clinically, the RBV dose reduction is performed based on the present Hb level. However, it has been noted that such dose adjustment does not effectively prevent the progression of anemia; that is, once a decrease of the Hb level has occurred, it is too late to stop the decline through RBV dose reduction, presumably because of erythropoietic delay.

In our subjects, the phosphorylated RBV level reached a steady-state by 4 weeks of RBV therapy ($1040 \pm 240\,\mu$M), implying gradual accumulation of phosphorylated RBV. Inoue et al. [8] reported that the erythrocyte phosphorylated RBV level at the steady-state at week 4 was $1218 \pm 234\,\mu$M and was well correlated with Hb reduction.

Table 2: Comparisons of the rate in which RBV was discontinued, the rate in which RBV dosage was reduced due to development of anemia, 4-week hemoglobin reduction rate from the baseline, the EVR rate and the SVR rate of the patients with 2-week erythrocyte phosphorylated RBV concentration <600 μM to 600–800 μM, and 800 μM $\leqq$.

	<600 μM ($n = 7$)	600–800 μM ($n = 7$)	800 μM $\leqq$ ($n = 10$)
Rate of RBV discontinuation (%)	0/7 (0)	0/7 (0)	3/10 (30.0)
Rate of RBV reduction (%)	1/7 (14.3)	3/7 (42.9)	3/10 (30.0)
ΔHb by 4 weeks (%)	-13.5 ± 8.8	-15.7 ± 6.5	-23.4 ± 11.6
Rate of EVR(%)	0/7 (0)	5/7 (71.4)	6/10 (60.0)
Rate of SVR (%)	0/7 (0)	3/7 (42.6)	3/10 (30.0)

RBV: ribavirin.
ΔHb: 4-week hemoglobin reduction rate from the baseline.
EVR: Early Virological Response; Serum HCV RNA negativity until 12 weeks of therapy.
SVR: Sustained Virological Response; Serum HCV RNA negativity at 24 weeks after completed therapy.
ΔHb is expressed as mean $\pm$ S.D.

However, as the Hb level had already decreased significantly by week 4, the RBV level at week 4 does not predict anemia. Therefore, we decided to evaluate whether the phosphorylated RBV level at week 2 might be useful for prediction of the subsequent development of anemia.

A close negative correlation was observed between the erythrocyte phosphorylated RBV level at week 2 and the ΔHb at week 4 in patients aged 50 years or over. In general, in elderly people, the percentage of fat cells in the bone marrow increases, and the reserve of marrow stem cells decreases with reduction of the hematopoietic mass and reduction in the ability for formation of erythroid colony-forming units [9, 10]. Additionally, RBV is known to be substantially excreted by kidney, and renal function decreases in elderly patients. Therefore, RBV accumulation is considered to be more likely to cause anemia in the elderly, especially due to the erythropoietic delay and the delay of RBV excretion. Nomura et al. reported that one of the higher risk of severe anemia was age higher than about 60 years [11].

In patients with RBV level of $\geq$800 μM at week 2, the ΔHb at week 4 was significantly higher, and a higher percentage of patients needed discontinuation of the RBV due to the development of anemia. Thus, we recommend that the erythrocyte phosphorylated RBV level be maintained at a level of less than 800 μM at week 2 for treatment safety.

The plasma RBV level has been reported not to be correlated with the SVR [12], but there have been few reports on the relationship between the erythrocyte phosphorylated RBV level and the treatment efficacy. The erythrocyte RBV level is about 150 times higher than the plasma RBV concentration, and most of the administered RBV is considered to be secreted into the urine. Of the proportion that remains in the body, most of it does accumulate as phosphorylated product within erythrocytes [13]. Furthermore, Homma et al. reported that little phosphorylated RBV existed in the plasma [13]. Since RBV taken up by cells is considered to be phosphorylated, and RMP and RTP are considered to have antiviral activities, the erythrocyte phosphorylated RBV level is a useful index for the antiviral effect of the drug. Since EVR, defined as a decrease of the HCV RNA level to 1/100 or zero at week 12 after the start of therapy, has been reported to be useful as a prognostic factor [14], we focused on not only SVR but also EVR.

In this study, the erythrocyte phosphorylated RBV level at week 2 was predictive of the EVR. Moreover, none of the patients in whom the phosphorylated RBV level at week 2 was <600 μM showed both EVR and SVR. Adjustment of the RBV dose to obtain an erythrocyte phosphorylated RBV level of $\geq$600 μM at week 2 is considered to be required to obtain an EVR. It would be necessary to have a large sample size, study of quality of life, and demonstration of better EVR and SVR in a prospective randomized trial. Recently, Fellay et al. reported that genetic variants leading to inosine triphosphatase (ITPA) deficiency protects against clinically significant decline in Hb level induced by HCV antiviral treatment [15]. We should examine the relationships between ITPA gene variants and RBV-induced anemia in Japanese populations, and evaluate the usefulness as an index to reduce the risk of anemia with erythrocyte RBV level.

5. Conclusion

In this study, the erythrocyte phosphorylated RBV level at week 2 is proposed as a useful indicator to determine an optimal dosage of ribavirin in patients with chronic hepatitis C under treatment with combination therapy with pegylated interferon and RBV.

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Hindawi Publishing Corporation
Hepatitis Research and Treatment
Volume 2010, Article ID 702748, 6 pages
doi:10.1155/2010/702748

Clinical Study

Differential Impact of Adherence to Pegylated Interferon and Ribavirin in the Treatment of Genotype 1 High Viral Titer Chronic Hepatitis C

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Received 3 May 2010; Revised 26 June 2010; Accepted 29 June 2010

Academic Editor: Emmet B. Keeffe

To clarify the impact of adherence, we treated 122 genotype 1 high viral titer chronic hepatitis C patients with pegylated interferon (peg-IFN) and ribavirin for 48 weeks at nine referral hospitals, and evaluated the prognostic factors with a focus on the adherence to the treatment. This study included 68 (55.7%) treatment-naïve patients and 54 (44.3%) patients who did not respond to the previous treatment. Multivariate analysis revealed adherence to peg-IFN and ribavirin as the only significant predictor. Sustained virological response (SVR) rate was 72.2%, 19.0%, and 27.3% in patients given ≥80%, 60%–80%, and <60% dose peg-IFN, respectively, and was 68.6%, 41.2%, and 5.3% in those given ≥80%, 60%–80%, and <60% dose ribavirin, respectively. SVR rate sharply fell when exposure to peg-IFN was below 80% whereas it decreased in a stepwise manner as for ribavirin. Therefore, ≥80% of peg-IFN and as much as possible dose of ribavirin are desired to achieve SVR in the treatment of genotype 1 high viral titer chronic hepatitis C.

1. Introduction

Although the combination of pegylated interferon (peg-IFN) and ribavirin (RBV) is the standard-of-care therapy for chronic hepatitis C, the sustained virological response (SVR) rate is still 40%–50% [1–3] for patients who are infected with genotype 1 and have high viral load in their sera. Adherence to the therapy is an important factor associated with a favorable outcome. McHutchison et al. reported that the patients who received ≥80% of the scheduled doses of peg-IFN and RBV for ≥80% of the planned duration of therapy had an SVR rate of 51% compared with 34% in less adherent patients [4]. In contrast, a study on patients with advanced fibrosis revealed that reducing RBV dose did not

affect SVR rate as long as peg-IFN dose was maintained [5]. Reddy et al. also reported that SVR rate was affected adversely by RBV dose reduction when cumulative exposure was less than 60%, and that RBV dose reduction raised the relapse rate [6]. The significant impact of adherence to both peg-IFN and RBV on SVR is well understood, however, there may be difference between these two drugs in the way they effect the response.

Until now, many host factors including younger age (40 years or less) [2], female gender [7], lighter body weight [1, 2], the absence of insulin resistance [8], elevated ALT levels [2], less advanced liver histology [2, 7], and non-African American race [7, 9] are reported to be associated with favorable response. Recently the association of genetic variation of *IL28B* with response has been reported [10–12].

Japanese elderly women were reported to be resistant to this therapy [13, 14]. Japanese patients are approximately 10 years older than those in other countries and our reports would provide useful information when considering therapy for elderly patients in other countries. The lower SVR rate in elderly women might be attributable to lower adherence to peg-IFN or RBV. However, few studies analyzed relationship between SVR rate and the adherence in elderly patients.

In this study, we treated genotype 1 high viral titer chronic hepatitis C patients with peg-IFN and RBV combination therapy, and evaluated the prognostic factors with a focus on the adherence to the treatment.

2. Materials and Methods

2.1. Patients. This study was performed at nine referral hospitals. Patients with hepatitis C virus (HCV) genotype 1 and high viral load (≥100,000 IU/mL) who received peg-IFN alfa-2b (Pegintron, Schering-Plough Corporation, Kenilworth, NJ) and RBV (Rebetol, Schering-Plough Corporation) combination therapy for 48 weeks from January 2004 to December 2006 were consecutively enrolled into the study. Exclusion criteria were as follows: (1) patients with leukopenia (<3,000/μL), neutropenia (<1,500/μL), thrombocytopenia (<90,000/μL), or anemia (hemoglobin concentration <12 g/dL), (2) patients with creatinine clearance <50 mL/min, and (3) existence of cirrhosis, autoimmune diseases, uncontrolled mental disorders, uncontrolled malignancy, or severe heart or lung diseases. Written informed consent was obtained from all patients.

2.2. Treatment. The patients were given peg-IFN alfa-2b at a dosage of 1.5 mg/kg every week subcutaneously for 48 weeks. Daily RBV was administered orally for 48 weeks according to the labeling approved by the Japanese Ministry of Health, Labour and Welfare; 600 mg for patients ≤60 kg, 800 mg for patients weighing 60 to 80 kg, and 1000 mg for patients >80 kg. The use of hematopoietic growth factors such as G-CSF and erythropoietin was not permitted in this study. Blood samples were collected every four weeks and parameters including complete blood cell counts, biochemistries, and the amount of HCV-RNA were determined. HCV serotype was tested with a serological genotyping assay kit (Immunocheck F-HCV Grouping; International Reagents Co., Tokyo, Japan) [15]. If HCV serotype was not definitive, HCV genotyping was performed (HCV Core Genotype; SRL, Tokyo, Japan). The response to the treatment was evaluated by an intention-to-treat analysis.

2.3. Statistical Analysis. The factors associated with SVR were analyzed by logistic regression using SPSS version 16 (SPSS Japan, Tokyo, Japan). Univariate or multivariate logistic regression analyses were performed to establish the factors contributing to SVR. All reported P-values are 2-sided, with $P < .05$ considered statistically significant. The difference in the rates of relapse or SVR was evaluated by chi-square test.

3. Results

3.1. SVR. A total of 122 patients were enrolled into the study. Forty-five patients (36.9%) were female and mean ± standard deviation (S.D.) of age was 54.0 ± 10.6 (min 19–max 70) years. Sixty-eight patients (55.7%) were naïve patients. The mean ± S.D. of weight and body mass index (BMI) was 63.5 ± 11.2 kg and 23.7 ± 3.3, respectively. High (100,000–800,000 IU/mL) and very high (≥800,000 IU/mL) HCV-RNA levels were observed in 36 (29.5%) and 86 (70.5%) patients, respectively. This study included 68 (55.7%) treatment-naïve patients and 54 (44.3%) patients who did not respond to the previous treatment. The previous treatment included a 24-week course of IFN alfa-2b and RBV combination therapy for 36 patients and a 24-week course of IFN alfa-2b or natural IFN alfa (human lymphoblastoid IFN) monotherapy for 18 patients. Forty-seven patients relapsed after the discontinuation of treatment, and the other 7 patients were nonresponders, in whom serum HCV-RNA were positive throughout the treatment. The SVR rate was 60.3%, 51.1%, and 28.6% in naïve patients, those with relapse, and nonresponders, respectively. In this study, the SVR rate was not significantly different between naïve patients and those treated previously. Liver biopsy was performed before treatment in 87 (71.3%) patients; 75 (86.2%) and 12 (13.8%) patients revealed METAVIR fibrosis score of 0–2 and 3-4, respectively. The SVR rate was not significantly different between these two groups; 57.3% in patients with F0–2 and 41.7% in those with F3-4. Finally 67 patients (54.9%) achieved SVR in the entire cohort.

3.2. Factors Associated with SVR (Table 1). Analyzed factors included gender, age, body weight, BMI, viral load, history of IFN treatment, and adherence to the treatment. Younger age, heavier weight, lower viral load, peg-IFN adherence, and RBV adherence were significant factors associated with SVR by univariate analysis. Multivariate analysis revealed adherence to peg-IFN and adherence to RBV as a significant predictor. We performed the same analysis after stratifying treatment-naïve and previously treated patients, and found adherence to peg-IFN and RBV as only factors significantly associated with SVR (data not shown) as shown in the entire cohort.

Patients given ≥80% dose of scheduled peg-IFN were more likely to achieve SVR by 7.7-fold (95% CI; 1.926–30.798, $P = .004$) than those given 60%–80% dose. The SVR

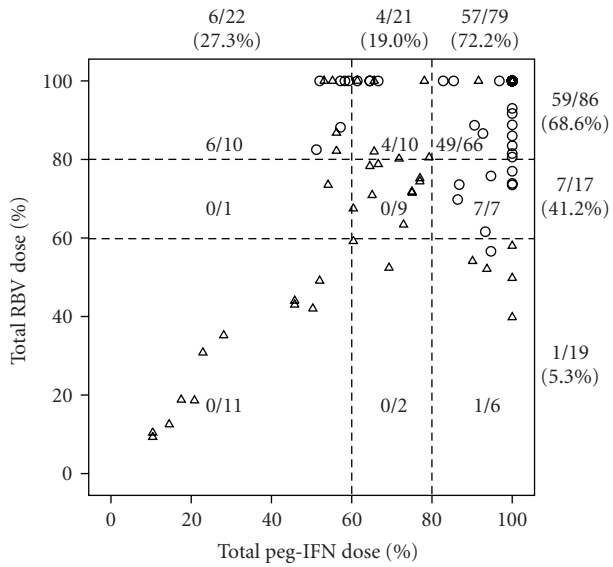

Figure 1: Scatter plot of patients with or without SVR according to administered total doses of peg-IFN and RBV. One hundred % represents a full scheduled dose. A circle and a triangle indicate a patient with SVR and one without SVR, respectively. A number represents number of patients with SVR/total number (SVR rate).

rate in patients given 60%–80% dose peg-IFN was similar with those given <60% dose. Patients given ≥80% dose and those given 60%–80% dose of scheduled RBV were more likely to obtain SVR than those given <60% by 27.4-fold (95% CI; 3.130–240.151, $P = .003$) and by 15.7-fold (95% CI; 1.289–190.653, $P = .031$), respectively. The outcome of each case was shown in a scatter plot (Figure 1). The SVR was 1/19 (5.3%), 7/17 (41.2%), and 59/86 (68.6%) in patients given <60%, 60%–80%, and ≥80% of total RBV dose, respectively (Figure 2). Therefore, the more RBV was administered, the higher was the SVR rate. On the other hand, SVR was achieved in 6/22 (27.3%), 4/21 (19.0%), and 57/79 (72.2%) patients given <60%, 60%–80%, and ≥80% of total peg-IFN dose, respectively. Peg-IFN dose of 80% or more was important to obtain SVR. Notably none of the patients who received <80% dose for both drugs resulted in SVR (Figure 1). The relationship between SVR and adherence was analyzed separately in the treatment-naïve group and the previously treated group. In the treatment-naïve group the SVR rate was 74.5%, 20.0%, and 36.4% in patients given ≥80%, 60%–80%, and <60% dose peg-IFN, respectively, and was 74.5%, 54.5%, and 0% in those given ≥80%, 60%–80%, and <60% dose RBV, respectively. In the previously treated group, SVR rate was 68.8%, 18.2%, and 18.2% in patients given ≥80%, 60%–80%, and <60% dose peg-IFN, respectively, and was 61.3%, 33.3%, and 11.1% in those given ≥80%, 60–80%, and <60% dose RBV, respectively. These trends were similar with the results obtained from the entire cohort.

There was a trend that younger patients received greater peg-IFN dose; 72/106 (67.9%) patients younger than 65 years and 7/16 (43.8%) patients aged 65 or older received ≥80% of total peg-IFN dose ($P = .059$).

Sixty-six patients (54.1%) received ≥80% dose for both drugs. Of these 49 (74.2%) patients resulted in SVR. When analysis was performed in these patients, no significant factors associated with SVR were chosen.

3.3. Rapid Virological Response (RVR), Early Virological Response (EVR), and Relapse. The population of patients whose serum HCV-RNA first disappeared at week 4 (RVR), week 8, week 12 (EVR), week 24, and week 48 was 10 (8.2%), 39 (32.0%), 28 (23.0%), 20 (16.4%), and 4 (3.3%) patients, respectively. Twenty-one (17.2%) patients were positive for HCV-RNA throughout the treatment period (null response). The SVR rate of these patients who became negative for HCV-RNA at week 4 (RVR), week 8, week 12 (EVR), week 24, and week 48 was 10/10 (100%), 35/39 (89.7%), 17/28 (60.7%), 5/20 (25%), and 0/4 (0%), respectively. In 101 patients negative for HCV-RNA at the end of treatment, 34 (33.7%) patients relapsed. Relapse rate was significantly lower in patients who received ≥80% dose of peg-IFN than that in those who received 60%–80% or <60% dose (18.6% in patients with ≥80% dose versus 69.2% in those with 60%–80% dose ($P < .001$) and 66.7% in those with <60% dose ($P < .001$), Figure 3). The relapse rate increased in a stepwise fashion according to the adherence to RBV (91.7% in patients with <60% dose versus 41.7% in those with 60%–80% dose ($P < .05$), and versus 23.4% in those with ≥80% dose ($P < .001$)). These results were inversely associated with SVR rates.

3.4. Adverse Effect. Seventeen (13.9%) patients discontinued treatment. The reasons of premature discontinuation were general fatigue and/or appetite loss (11 patients), fundal hemorrhage (1 patient), deterioration of diabetes mellitus (1 patient), and depression (1 patient). Three patients discontinued treatment because of positive HCV-RNA at week 24. Thirty-nine (32.0%) and 33 (27.0%) patients required dose reduction of peg-IFN and RBV, respectively. Major reasons of dose reduction were neutropenia or thrombocytopenia for peg-IFN and anemia for RBV. Common adverse effects included general fatigue, appetite loss, weight loss, and pruritus. In 12 patients with advanced liver disease (METAVIR fibrosis score of 3-4), 6 (50%) and 4 (33.3%) patients required dose reduction of peg-IFN and RBV, respectively. In 75 patients with milder liver disease (METAVIR fibrosis score of 0–2), 22 (29.3%) and 20 (26.7%) patients required dose reduction of peg-IFN and RBV, respectively. There was no significant difference between these two groups in the proportion of patients who required dose reduction.

4. Discussion

The mean age of our study population was 54.0 years, which was approximately 10 years older than patients of major studies in Western countries [1–3]. Our cohort consisted of treatment-naïve patients (55.7%) and those who did not respond to the prior treatment (44.3%). SVR was achieved in 54.9% patients.

In our study, adherence to peg-IFN and RBV was the only significant factor associated with SVR. Interestingly,

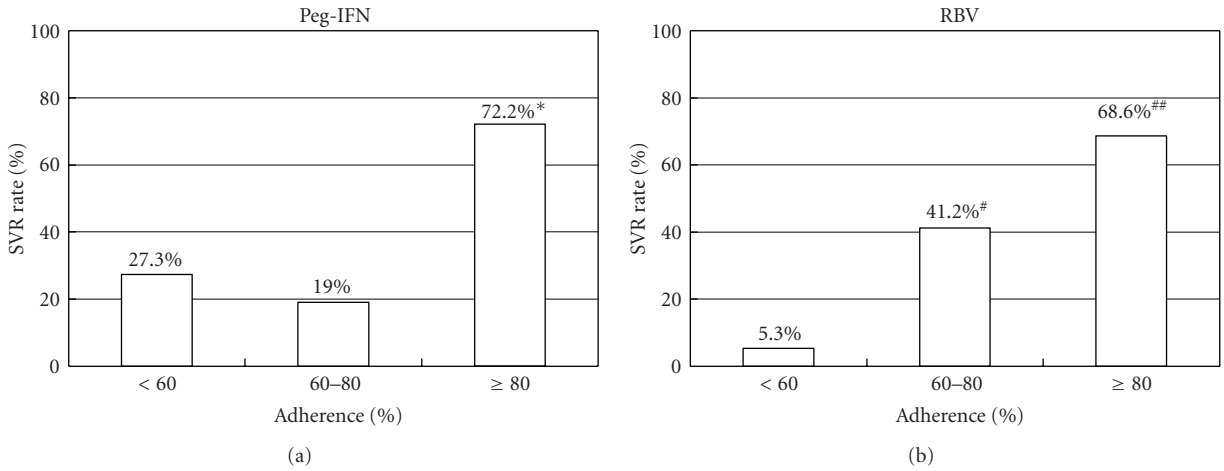

Figure 2: SVR rates classified by adherence to peg-IFN and RBV. *The SVR rate in patients with ≥80% dose of peg-IFN was significantly higher than that in those with <60% and 60%–80% ($P < .001$ for both). #The SVR rate in patients with 60%–80% dose of RBV was significantly higher than that in those with <60% ($P < .05$). ##The SVR rate in patients with ≥80% dose of RBV was significantly higher than that in those with <60% ($P < .001$) and 60%–80% ($P < .05$).

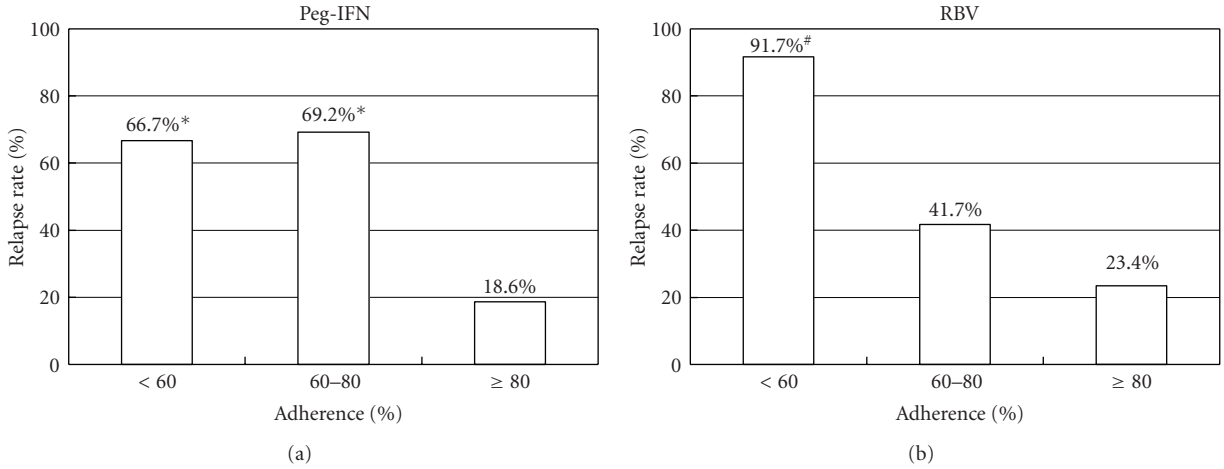

Figure 3: Relapse rates classified by adherence to peg-IFN and RBV. *The relapse rate in patients with <60% and 60%–80% dose of peg-IFN was significantly higher than that in those with ≥80% ($P < .001$ for both). #The relapse rate in patients with <60% dose of RBV was significantly higher than that in those with 60%–80% ($P < .05$) and ≥80% ($P < .001$).

the SVR rate stepwisely rose by the increase of administered dose of RBV. In contrast, 80% or more dose of peg-IFN was required to achieve SVR (Figure 2). This observation resulted from the likelihood of relapse (Figure 3); higher relapse rate was documented in a stepwise fashion in patients with smaller exposure to RBV, as previously suggested [16–18]. SVR rate was 74.2% when both drugs were administered ≥80%. Notably none of the patients who received <80% dose of both drugs attained SVR (Figure 1), confirming the validity of 80/80/80 rule together with ≥80% treatment duration.

The difference between peg-IFN and RBV in the impact of adherence on SVR, especially within the <80% dose range, is still unclear. In our study, SVR rate sharply fell when exposure to peg-IFN was below 80% whereas it decreased in a stepwise manner as for RBV. Hiramatsu et al. recently reported that RBV dose reduction raised relapse rate in a dose-dependent manner [19], which is in agreement with our results.

At least 80% dose of peg-IFN will be necessary to obtain favorable outcome. In contrast, RBV should be administered as much as possible within the planned dose. To accomplish this, RBV dose should be reduced by 200-mg decrements when anemia appears, and restored to the previous dose when anemia improves. Higher than standard dose RBV given together with standard dose peg-IFN may increase SVR rate [20], however, safety issues such as severe anemia are the major concern for this approach. Although the use of erythropoietin contributes to maintain RBV dose, the effect on SVR has not been shown [21, 22].

Table 1

Variables	SVR	*P*-value	Adjusted OR (95% C.I.)	*P*-value
Sex		.091		.501
Female	20/45 (44.4%)		1.00	
Male	47/77 (61.0%)		1.429 (0.506–4.032)	
Age (yr)		.019		.398
65<	5/16 (31.3%)		1.00	
51< ≤64	35/68 (51.5%)		2.655 (0.581–12.132)	
≤50	27/38 (71.1%)		2.695 (0.574–12.659)	
Weight (Kg)		.028		.116
<65	31/68 (45.6%)		1.000	
65≤	36/54 (66.7%)		3.053 (0.760–12.274)	
BMI		.716		.158
24≤	30/57 (52.6%)		1.000	
<24	37/65 (56.9%)		2.747 (0.674–11.236)	
Viral load (IU/mL)		.015		.174
800,000≤	41/86 (47.7%)		1.000	
100,000≤ <800,000	26/36 (72.2%)		2.137 (0.716–6.369)	
History of IFN treatment		.203		.581
yes	26/54 (48.1%)		1.000	
no	41/68 (60.3%)		1.316 (0.496–3.493)	
Peg-IFN adherence (%)		<.001		.008
<60	6/22 (27.3%)		2.637 (0.448–15.513)	.284
60≤ <80	4/21 (19.0%)		1.000	—
80≤	57/79 (72.2%)		7.702 (1.926–30.798)	.004
RBV adherence (%)		<.001		.010
<60	1/19 (5.3%)		1.000	—
60≤ <80	7/17 (41.2%)		15.679 (1.289–190.653)	.031
80≤	59/86 (68.6%)		27.416 (3.130–240.151)	.003

Sezaki et al. reported that elderly women were resistant to peg-IFN and RBV combination therapy in Japan [13, 14]. In our study, younger age was a significant factor by univariate analysis, however, neither gender nor age was significantly associated with SVR by multivariate analysis. There was a trend towards lower adherence to peg-IFN in elderly patients. Therefore, older age itself is not a significant factor but is related to dose reduction or discontinuation, as reported by Iwasaki et al. [23].

SVR rate was 74.2% when both drugs were administered ≥80%. Japanese patients are approximately 10 years older than those in other countries and anticipated to be vulnerable to adverse effects. Therefore, the adjuvant therapy that alleviates adverse effects should be developed. We recently demonstrated that maloxicam, a COX-2 inhibitor, decreased the rate of patients who required dose reduction by preventing the decrease of neutrophil counts [24].

In this study, serotyping was used instead of genotyping because genotyping was not covered by the Japanese national health insurance. Serotype 1 includes genotype 1a and 1b. Because genotype 1a is rarely observed in Japan [25], most patients of this study are assumed infected with genotype 1b. Limitation of this study is a retrospective analysis with relatively small number of patients. Other major limitations are that our study consisted of a heterogeneous cohort (treatment-naïve and previously treated patients) and that liver histology was not available in approximately one third of the patients.

In conclusion, 80% or more dose of peg-IFN and as much as possible dose of RBV are desired to achieve SVR in the treatment of genotype 1 high viral titer chronic hepatitis C.

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Hindawi Publishing Corporation
Hepatitis Research and Treatment
Volume 2010, Article ID 537827, 5 pages
doi:10.1155/2010/537827

Clinical Study

Retreatment of Patients Nonresponsive to Pegylated Interferon and Ribavirin with Daily High-Dose Consensus Interferon

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Received 17 April 2010; Revised 21 July 2010; Accepted 4 August 2010

Academic Editor: Tatehiro Kagawa

Background. Current treatment of chronic hepatitis C with pegylated interferon and ribavirin has the ability to eliminate viral infection in about half of the patients treated. Therapeutic options, for those with remaining chronic hepatitis, will remain limited until novel antivirals become available in the future. Consensus interferon is currently available and has demonstrated clinical efficacy with superior *invitro* antiviral activity, but the maximum tolerated dose is not defined. *Methods*. We assessed the efficacy of daily high-dose (24 ug) consensus interferon with weight-based (1000–1200 mg daily) ribavirin in HCV genotype 1-infected non-responder patients. *Results*. Six adverse events were documented in five patients, and the trial was terminated with no subject achieving viral clearance. *Conclusions*. The occurrence of serious adverse events effectively defined the upper limit of acceptable dose, while also revealing that this dose did not offer enhanced sustained viral clearance.

1. Introduction

The most commonly employed treatment of patients with hepatitis C virus (HCV) infection who are treatment-naïve consists of pegylated interferon-alfa and ribavirin with sustained viral response (SVR) rates in genotype 1 patients of up to 52% [1, 2]. Since the FDA approval of pegylated interferon and ribavirin, there are an ever-increasing number of hepatitis C patients who have failed to respond to this combination therapy. There is no standard of care for the treatment of patients who have failed to respond to pegylated interferon and ribavirin [3]. Consensus interferon (CIFN), in combination with ribavirin, has recently been approved for this use. Patients with advanced fibrosis may not have time to await development of future antiviral agents and may need to utilize currently available therapies.

CIFN has more potent antiviral *invitro* activity compared to other interferons [4, 5]. Currently, CIFN is approved for initial or retreatment of persons infected with HCV. Recent clinical trials report reasonable SVR rates (23% to 37%) in the treatment of nonresponders to standard or pegylated interferon and ribavirin using different regimens of daily CIFN and weight-based ribavirin dosage [6–8]. Cornberg et al. found that an eight-week induction phase with CIFN doses of 18 μg followed by 9 μg for 40 weeks did not result in a higher SVR compared to 9 μg for 48 weeks, presumably due to dose modifications in subjects receiving the induction dose of CIFN. When the subgroup of patients receiving the higher dose of CIFN, without early dose modification, was evaluated, 40% achieved SVR [8]. There was one multicenter randomized control trial, the DIRECT trial, evaluating the efficacy of daily CIFN at doses of 9 μg or 15 μg with weight-based ribavirin administration in the treatment of patients unresponsive to prior pegylated interferon and ribavirin treatment. Viral clearance was achieved in a dose-dependent manner with greatest loss of HCV in the 15 μg group [9].

Although this trial suggested a dose-dependent rate of viral clearance, there has been no dose-ranging information to set the maximum tolerated dose of daily CIFN in this difficult-to-treat group of hepatitis C patients. Given the urgent need of treatment for patients with advanced fibrosis and limited access to antiviral agents under development, evaluation of available agents in novel dosing regimens is needed.

The aim of our clinical trial was to evaluate the efficacy, safety and tolerability of high-dose daily CIFN (24 μg) with weight-based ribavirin dosage given with maximal support of patient adherence including blood cell growth factors. Previous clinical trials did not evaluate this high dose of daily CIFN in the treatment of non-responders to pegylated interferon and ribavirin beyond an 8-week induction period [9]. Since the previous data have suggested a possible dose-dependent response to daily CIFN and ribavirin, determining the utility of a higher dosage of daily CIFN with weight-based ribavirin will be important in this group of hepatitis C patients with few efficacious available treatment options.

2. Methods

Eligible subjects for our open-label trial were patients infected with chronic hepatitis C genotype 1 previously treated with either pegylated interferon alfa-2a or alfa-2b and ribavirin. In addition, the subjects must have been nonresponsive to prior treatment, defined as the lack of a 2-log drop of hepatitis C viral ribonucleic acid (HCV RNA) from baseline at 12 weeks of therapy or the presence of detectable HCV RNA at 24 weeks of therapy. All subjects must have previously received a weight-based dosage of ribavirin. At least one liver biopsy, consistent with chronic hepatitis C without significant alternative liver disease, must have been performed prior to screening for enrollment into the trial. All subjects, prior to participation, signed a consent form approved by each institutional review board.

This study was an open-label pilot trial. All study subjects were treated with a daily 24 μg dose of CIFN (Infergen-Valeant Pharmaceuticals, Costa Mesa, CA) given subcutaneously and a daily weight-based dose of ribavirin (1000–1200 mg per day). All patients were evaluated at weeks one, two, and four of therapy and at subsequent four week intervals unless additional urgent clinical evaluation was needed. At each visit, subjects underwent physical examination and clinical history detailing adverse events and completion of the Beck Depression Inventory second edition (BDI-II). Treatment was discontinued and the subject considered a treatment failure if the patient failed to achieve early virologic response (EVR) defined as less than a 2-log drop of HCV RNA from baseline at Week 12 of therapy, or had detectable HCV RNA at or after Week 24 of therapy. Sustained viral response (SVR) was defined as an undetectable HCV RNA level using Quest Diagnostics (Madison, NJ) Heptimax and TMA serum assays 24 weeks after completion of 48 weeks of treatment.

Potential subjects for this clinical trial were recruited from three hepatology practices in New York. Compliance with the experimental protocol was assessed by the subjects' self-reported administration of ribavirin and the return of used CIFN vials. In addition, the trial had a data safety monitor (DSM), who assessed the safety of this trial at specified time points during the study. The DSM was an independent physician with extensive clinical experience with treatment of patients with hepatitis C infection.

The subject's baseline demographics were compared using Student's t-test, chi-square and Fisher's exact tests. Response rates between partial and null responders were compared using chi-square and Fisher's exact tests.

Table 1: Demographics and baseline data.

Patient Characteristic	Results
Ethnicity:	
Caucasian	61.5%
Hispanic	30.8%
African-American	7.7%
Weight (kilograms)	
Mean ± SEM	84.0 ± 14.9
Histology*	
Stage 1-2	3
Stage 3	5
Stage 4	5
Response to prior treatment (n)	
Null response	10
Partial response	3
WBC count (k/μL)	
Mean ± SEM	0.3 ± 1.5
Hemoglobin (g/dL)	
Mean ± SEM	14.6 ± 1.4
Platelet count (k/μL)	
Mean ± SEM	194.9 ± 68.0

*Fibrosis stage defined as modified Ishak score determined as defined by Theise [10].

3. Results

Thirteen subjects (8 men) with a mean age of 55-years were enrolled in this study. The median baseline HCV RNA level was 3,300,000 IU/mL with only two subjects with levels less than 1,000,000 IU/mL. Ten patients had advanced fibrosis (Table 1). During prior initial therapy, ten of the subjects had failed to achieve loss of HCV RNA by 12 weeks of therapy. Three subjects had shown a 2-log decrease in HCV RNA by 12 weeks but had remained HCV RNA detectable at 24 weeks of therapy.

The trial was terminated early due to the occurrence of serious adverse events ($n = 6$) in five patients. These serious adverse events included abdominal wall abscess requiring hospitalization, severe dehydration, and hypotension resulting in metabolic derangements, loss of consciousness, anemia, and ventricular tachycardia. In addition, one subject discontinued therapy due to disabling fatigue. The trial had initially been planned to enroll thirty subjects at this interferon dose. The study termination decision was made by the Principal Investigator on the advice of the DSM after assessing the frequency of serious toxicity while no patient

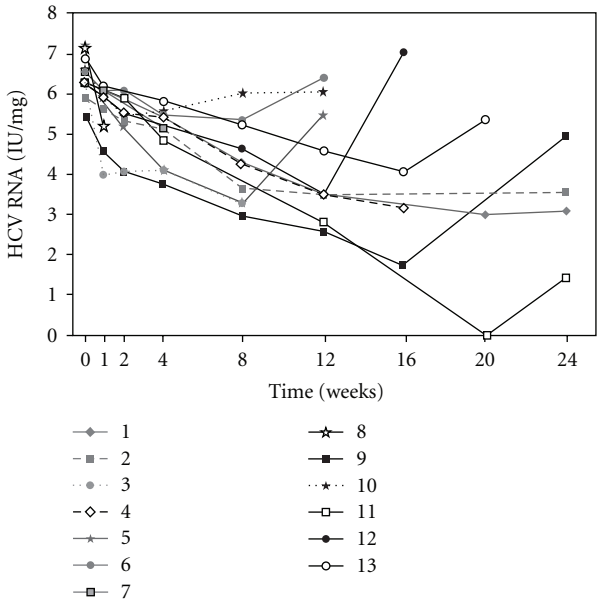

Figure 1: The graph shows the viral kinetics of the 13 subjects during treatment with CIFN and weight-based ribavirin. None of the subjects with an initial drop in HCV RNA, including subject 11 who had transient undetectable HCV RNA at week 20, failed to achieved a sustained loss of HCV RNA.

had achieved a sustained loss of HCV RNA. The severity and frequency, of serious adverse events, are evidence that the high-dose aggressive treatment regimen of this study defined an upper dose limit of tolerability for CIFN-ribavirin therapy.

Subjects were discontinued from treatment, according to protocol, when therapy was judged to be futile or for adverse events. Only four subjects received 24 weeks of treatment. The median maximal decrease of HCV RNA from baseline on treatment was 3, 135, 000 IU/ml. Nine subjects (69%) had greater than a 2-log drop from baseline HCV RNA while on treatment. One of these had transient loss of HCV but subsequently experienced a viral breakthrough. The four subjects who received 24 weeks of therapy all had partial viral response after 12 weeks of therapy, but had detectable hepatitis C virus at week 24 and therapy was discontinued as they were deemed non-responders (Figure 1).

Three subjects with partial viral response prematurely discontinued therapy due to serious adverse events. There were three subjects still on therapy when the trial was halted. One subject had greater than a 2-log drop in HCV RNA level from baseline by week 8 of therapy, but the HCV RNA level at the time of termination had markedly increased, and there was no longer a 2-log drop in the HCV RNA level from baseline at that point. The second subject did have a greater than 2-log decrease from baseline HCV RNA level after 16 weeks of therapy, but by 20 weeks the hepatitis C viral level had increased over 1-log from the nadir level. The third subject did not have a 2-log decline from baseline viral level after 12 weeks of therapy and was deemed a non-responder. The final three subjects were non-responders to the study medications at the time of discontinuation of therapy due to adverse events.

There were three subjects with greater than a 2-log drop in viral level by four weeks of therapy. One of these subjects could not tolerate therapy and did not complete 12 weeks of therapy. Another was a nonresponder at 24 weeks of therapy. The third subject was still on study medication when the clinical trial was halted, however, while on therapy there was marked increase in HCV RNA level. All three subjects who had greater than a 2-log drop from the baseline viral level after four weeks of therapy were partial responders to previous pegylated interferon and ribavirin therapy compared to none of the prior null responders ($P < .04$).

The most common adverse events were fatigue ($n = 12$) and flu-like symptoms ($n = 12$), anorexia ($n = 10$), anxiety/depression ($n = 7$), and insomnia ($n = 7$). The mean decrease in body weight during the experimental protocol was 8.1 ± 5.8 kilograms. The median increase in BDI-II score was 9 ± 10.1. Serious adverse events ($n = 6$) occurred in five patients and included abdominal wall abscess, severe dehydration, hypotension, and ventricular tachycardia all requiring hospitalization and loss of consciousness and anemia. These events occurred within the first six to 16 weeks of therapy. Two subjects (15%) had their dose of CIFN reduced during the trial from 24 μg to 15 μg daily. Five subjects (38.5%) had a dose reduction of ribavirin and one of the five subjects had ribavirin discontinued. The mean decrease in hemoglobin level from baseline to nadir was 3.6 ± 1.6 g/dL with eight subjects receiving epoetin alpha injections.

No subject had a decrease in absolute neutrophil count below 500/μL. The mean decrease in WBC level from baseline to nadir was $4,200 \pm 1,300/\mu$L. The mean decrease in platelet count from baseline was $104,000 \pm 5,200/\mu$L.

4. Discussion

Almost half the patients with HCV genotype 1 infection are left with residual infection following treatment with pegylated interferon and ribavirin. Evaluation of currently available agents to optimize therapeutic efficacy is important, particularly for patients with advanced hepatic fibrosis.

Previous studies demonstrate promising SVR rates of 23% to 37% using daily CIFN and ribavirin in the retreatment of non-responders to the combination of standard or pegylated interferon and ribavirin [6–8]. HCV clearance by CIFN appears to be a dose-related response [8, 9, 11]. The efficacy of high-dose CIFN, however, may be limited by tolerability and dose reductions for adverse events. There is no study defining the dose-limiting toxicity of CIFN used with ribavirin. One study, by Böcher et al., compared the efficacy of CIFN and ribavirin with high-dose induction (9 μg daily for 24 weeks followed by 9 μg three times per week for 24 weeks) to lower-dose treatment (CIFN 18 μg three times per week for 12 weeks, followed by 9 μg three times per week for 36 weeks) in those patients who had previously failed treatment with interferon and ribavirin therapy. The week 12 and 24 responses were superior in the high- versus

low-dose group ($P < .05$), but the SVR rates were identical at 26%. This suggests that the initial positive effect of the 24 weeks of high-dose daily induction was lost after dose reduction to three times per week treatment [12].

Kaiser etal. from Germany evaluated an induction dose of daily CIFN of 27 μg for four weeks followed by 18 μg for eight weeks versus 18 μg for 12 weeks in 120 patients infected with HCV who were previous non-responders to pegylated interferon and weight-based ribavirin [6]. No ribavirin was given during the induction time period. The SVR rate was 44% in the high-dose group compared to 39% in the lower-dose group. The rate of discontinuation of therapy was 10% in the higher dose arm, and CIFN dose was reduced in 17%. Since this study required significant dose reductions without blood cell growth factor support, the ability to assess the efficacy potential of high-dose daily CIFN was limited.

Another study by Cornberg and colleagues investigated the efficacy of CIFN plus ribavirin in HCV patients who were non-responders to standard interferon and ribavirin [8]. Consensus interferon dosing with 18 μg for the first 8-weeks of treatment resulted in an enhanced first-phase HCV-RNA decay suggesting higher antiviral efficacy of a higher dose of CIFN, but this did not translate to a better SVR, presumably due to dose modifications [8]. Based on the suggestion of a dose-dependent response and a superior response to high-dose CIFN, our trial sought to determine the upper limit of tolerability of CIFN dose in these difficult-to-treat patients.

Previous partial responders to pegylated interferon and weight-based ribavirin as compared to null responders had better viral kinetic responses with all three partial responders having greater than a 2-log drop in HCV RNA from baseline viral level by week four. This response, however, did not translate into a higher overall efficacy since these subjects could not tolerate continued therapy and viral response did not decline to undetectable levels.

This trial used a sustained high daily dose (24 mg) of CIFN, a treatment regimen which showed promising early results in achieving a decline in HCV RNA levels in a population of subjects resistant to prior therapy. The major factor leading to termination of this study was the occurrence of serious adverse events. Although each event was not unique in patients receiving interferon ribavirin treatment, the occurrence of frequent serious adverse events in a small patient population raised significant concern. The abdominal wall abscess was related to injection site infection. The development of dehydration and hypotension was related to anorexia and severe lassitude. The episode of loss of consciousness may have been related to anorexia and anemia. The observed adverse events, even including cardiac arrhythmias, have been reported previously in patients receiving interferon alfa and ribavirin. The frequency and severity of adverse events, however, effectively determined the upper limit of tolerability of daily consensus interferon to be less than the 24 μg daily dose when used in combination with weight-based ribavirin. This observation is important since the efficacy results of the prior studies left open the possibility of greater efficacy of high-dose CIFN.

Despite use of blood cell growth factors and maximal support of experienced investigators, this high-dose of daily CIFN therapy in combination with a weight-based ribavirin dose was not tolerated due to serious adverse events. Most of the subjects required epoetin alfa injections for therapy-associated anemia. Even in the subjects who could tolerate high-dose CIFN therapy with weight-based ribavirin, sustained loss of HCV was not achieved by 24 weeks.

We cannot recommend this higher daily 24 μg dose of CIFN in combination with weight-based ribavirin. Our study effectively defines the upper limit of tolerability of daily CIFN dosage when used in combination with ribavirin. Furthermore, this high dosage of CIFN and weight-based ribavirin combination treatment was ineffective in eradicating hepatitis C virus in patients who failed previous pegylated interferon and ribavirin therapy.

Abbreviations

Cifn:	consensus interferon
HCV:	hepatitis C virus
SVR:	sustained viral response
HCV RNA:	hepatitis C viral ribonucleic acid
DSM:	data safety monitor
WBC:	white blood cell
BDI-II:	Beck Depression Inventory second edition.

Author Disclosures

Dr. Henry C. Bodenheimer, Jr consults for Vertex and Novartis and is on an advisory board for Roche Genentech. Ivanka Zic, R. N., MSN was employed by Beth Israel Medical Center at the time of this research study. She is now employed by Merck.

Acknowledgment

This study was conducted using funds from Inter-Mune (Brisbane, Calif., USA) given to Beth Israel Medical Center, NY, as an unrestricted research grant.

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Hindawi Publishing Corporation
Hepatitis Research and Treatment
Volume 2010, Article ID 961359, 4 pages
doi:10.1155/2010/961359

Review Article

Hepatitis C in Haematological Patients

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Received 19 April 2010; Revised 8 July 2010; Accepted 6 August 2010

Academic Editor: Tatehiro Kagawa

There is no consensus guideline concerning the management of chronic hepatitis C patients during chemotherapy, and immunosuppression. However, there are some suggestions in literature that hepatitis C viral load increases during chemotherapy and there is a risk of rebound immunity against hepatitis C after discontinuation of immunosuppression with a consequent liver injury. A close monitoring of liver function of these patients is prudent during treatment of haematological malignancy. Antiviral treatment is deferred after the completion of chemotherapy and recovery of patients' immunity to minimize the toxicity of treatment. A combination of pegylated interferon and ribavirin is the standard therapy in hepatitis C infected haematological patients.

1. Introduction

It is well known that reactivation of hepatitis B is a potential lethal complication of chemotherapy and prophylactic antiviral drugs are prudent during treatment of haematological malignancies. However, on the contrary, it is not certain how chronic hepatitis C infection would affect the outcome of haematological malignancies and bone marrow transplantation patients. There are case reports of severe flare up of chronic hepatitis C in patients undergoing chemotherapy [1, 2] but case series review found such complications uncommon [3]. On the other hand, long-term survivors of bone marrow transplantation recipients are more prone to the complications of hepatitis C infection [4] and treatment in this group of patients seems warranted. However, there is scanty data on management of chronic hepatitis C in haematology patients.

Hepatitis C is associated with development of haematological diseases ranging from immune thrombocytopenia to lymphoma. The underlying pathogenesis and its impact on treatment are discussed.

2. Hepatitis C

There are six major genotypes of hepatitis C and more than 50 subtypes. Genotype 1b is the most common subtype worldwide. About 170 million people have chronic hepatitis and the estimated annual incidence of new case of hepatitis C is 3 to 4 millions [5]. In a study in Europe, the approximate prevalence of chronic hepatitis C among bone marrow transplantation recipients is 6% [6].

Sixty to eighty percent of patients develop chronic hepatitis C after acute infection. About twenty percent of these patients will be complicated by cirrhosis in twenty to thirty years, and some of them may develop hepatocellular carcinoma [5, 7]. It is believed that successful control of viral replication by effective antiviral treatment would prevent such complications in these patients [8, 9].

3. Status of Hepatitis C during Chemotherapy and Immunosuppression

The level of hepatitis C viral RNA in blood has been shown to increase during chemotherapy and immunosuppression. At the same time, for those with pre-existing liver dysfunction, the transaminase levels often normalize during immunosuppression. Upon the withdrawal of chemotherapy or immunosuppressants, the hepatitis C viral RNA decreases with a concomitant rise in transaminase levels. In a study of ten chronic hepatitis C patients, the alanine transaminase level decreased in eight patients while all of them

demonstrated a rise in hepatitis C RNA during a seven-week course of prednisone whereas there was a rebound of alanine transaminase upon the withdrawal of steroid in seven of them [10]. None of these patients developed fulminant hepatitis. Similarly, in another study of lymphoma patients who received rituximab with combination chemotherapy, the hepatitis C RNA increased during chemotherapy and declined after completion of treatment. The use of rituximab in patients without hepatitis seldom leads to hepatotoxicity. However, in this cohort, one patient developed significant hepatotoxicity during rituximab chemotherapy [11].

This fluctuation in hepatitis C viral load and liver enzymes during and after chemotherapy or immunosuppression is explained by the suppression of immunity during chemotherapy and a rebound of reaction towards hepatitis C upon its withdrawal.

However, despite the above observations, the clinical impact of chronic hepatitis C infection on patients undergoing bone marrow transplantation or chemotherapy is not well characterized. In a study in United States, among thirty three chronic hepatitis C patients, only eighteen of them (55%) developed mild to modest elevation of liver enzymes during chemotherapy [3]. None of them developed severe flare up that required cessation of chemotherapy. On the contrary, a study in Europe reported that up to 65% of the patients with chronic hepatitis C infection developed significant hepatotoxicity during chemotherapy [12]. This high incidence of hepatotoxicity led to interruptions in chemotherapy and jeopardized the clinical outcome of these patients. In another study of 132 patients, five patients had to discontinue chemotherapy because of severe hepatic dysfunction during treatment [13]. In a recently published review of 160 chronic hepatitis C patients with non-Hodgkin lymphoma, twenty-four (15%) of them developed significant liver toxicity during chemotherapy [14]. The overall survival of chronic hepatitis C patients is also shown to be significantly worse than those without hepaitits C infection. At a median followup of two years of patients diagnosed with diffuse large B cell lymphoma in Groupe d'Etude des Lymphomes de l'Adulte (GELA) programs, the overall survival was 56% among chronic hepatitis C patients versus 80% in those without ($P = .02$) [12].

The reported incidence of hepatotoxicity in chronic hepatitis C patients undergoing chemotherapy varies greatly among these studies. In addition, because of the bleeding tendency commonly seen in haematology patients, all these studies only monitored liver enzymes and hepatitis C RNA level but none of them was based on histological evidence. Therefore, there is yet concrete data on the effect of chronic hepatitis C infection in patients receiving chemotherapy and more studies are needed in this aspect.

4. Hepatitis C in Hematopoietic Stem Cell Transplantation

Hepatitis C infection is associated with an increased risk of veno-occlusive disease (VOD) and graft-versus-host disease (GVHD) of liver. The reported incidence of VOD was 14% among those with chronic hepatitis C infection while it was only 8% in transplant recipients who were negative for hepatitis C. The chronic inflammation in hepatitis C-infected liver causes endothelial changes in the hepatic sinusoids and this may predispose the patients to VOD during bone marrow transplant [15].

Although significant liver dysfunction during or immediately posttransplant is uncommon, bone marrow transplantation recipients with chronic hepatitis C infection have a significantly worse long-term outcome. The estimated incidence of cirrhosis at twenty years post bone marrow transplantation is 24% in those with chronic hepatitis C [16]. Moreover, there is evidence that the annual fibrosis progression rate is significantly higher in posttransplantation patients than those chronic hepatitis C patients without transplantation [4]. In fact, chronic hepatitis C infection ranked the third as a cause of late mortality, after infections and GVHD in posttransplant patients [16]. Once they developed cirrhosis, their survival outcomes are markedly compromised.

5. Treatment of Hepatitis C in Haematological Patients

The current standard of treatment of chronic hepatitis C infection is a combination of pegylated interferon and ribavirin. There is currently no specific guideline for treatment of the infection in haematological malignancy patients. In these patients, including bone marrow transplantation recipients, hepatitis C treatment is deferred until patients' immunity and bone marrow recover. In particular, for allogeneic stem cell transplantation patients, hepatitis C treatment should be withheld until all immunosuppressants are tailed off and GVHD is completely resolved. Interferon is known to suppress bone marrow hematopoiesis and this would aggravate the cytopenia frequently seen in postbone marrow transplantation patients. Moreover, it is reported that the use of interferon in allogeneic bone marrow transplantation patients might trigger GVHD, and its use in stem cell recipients should be cautious [17]. Ribavirin may suppress erythropoiesis and this may aggravate the degree of anemia in hematology patients. The use of erythropoietin however, is shown to reduce the transfusion requirement of these patients.

Combination therapy of pegylated interferon and ribavirin is shown to produce a sustained virological response, that is, undetectable hepatitis C RNA six months off therapy, in 20% of chronic hepatitis C patients after bone marrow transplantation. This response rate is compared unfavourably with that reported for the general population. Moreover, 30% of the studied patients did not receive this treatment because of the presence of contraindications [18]. The poor tolerability among hematology patients and their inferior treatment outcome warrant more research in this particular group of patients.

6. Hepatitis C and Autoimmune Cytopenia

Chronic hepatitis C infection is associated with thrombocytopenia, and the cause is multifactorial in most patients.

There are various possible explanations, which include increased sequestration and destruction of platelets in patients with hypersplenism, reduced thrombopoiesis as a result of decreased production of endogenous thrombopoietin by diseased liver, direct marrow suppression by hepatitis C virus as well as dysregulation of immunity leading to autoimmune thrombocytopenia. One study demonstrated the presence of increased level of antiplatelet glycoproteins in serum of chronic hepatitis C infected patients with thrombocytopenia [19]. A significant proportion of these patients responded to standard immune thrombocytopenia therapy such as steroids and intravenous immunoglobulin. In two of the studied patients, the platelet count normalized after receiving pegylated interferon plus ribavirin therapy. The rise of platelet count in one of these patients coincided with the disappearance of hepatitis C RNA in blood [19]. Although it is well known that interferon causes cytopenia, its administration in hepatitis C infected patients with cytopenia is not necessarily contraindicated but should be evaluated on an individual basis.

7. Hepatitis C in Lymphomagenesis

It is well known that hepatitis C is a lymphotropic virus and is able to infect mononuclear cells in peripheral blood. Chronic hepatitis C infection is associated with various lymphoproliferative disorders and the most commonly reported association is mixed essential cryoglobulinemia. More than 95% of patients with mixed cryoglobulinemia have evidence of exposure to hepatitis C virus [20, 21].

B-cell clonality and t(14;18) translocation are both prevalent in the peripheral blood mononuclear cells of hepatitis C infected patients [22]. It has been shown that chronic hepatitis C infection is significantly associated with the development of non-Hodgkin lymphoma. The reported odds ratio ranged from 2 to 4, with the risk being more evident in area with a higher prevalence of hepatitis C infection [23]. The most commonly reported subtypes of non-Hodgkin lymphoma in chronic hepatitis C infected patients are marginal zone lymphoma and lymphoplasmacytic lymphoma. There are also reports on an increased prevalence of high grade B-cell lymphoma in hepatitis C infected patients but majority of them were arising from an underlying low-grade lymphoma.

It is found that CD81 is a hepatitis C coreceptor and it is expressed in B lymphocytes [24]. The engagement of CD81, which is part of the CD81/CD19/CD21 membrane complex, activate B lymphocytes and subsequently lead to their proliferation. As a result, this chronic antigenic stimulation may predispose to the development of B-cell lymphoproliferative disorders [25]. On the other hand, there are suggestions that chronic hepatitis C infection induces point mutations in both immunoglobulin and nonimmunoglobulin genes of infected B lymphocytes [26, 27]. It is, however, still controversial whether hepatitis C virus has a direct oncogenic effect. More studies are warranted before a definitive conclusion can be made.

8. Role of Antiviral Treatment in Hepatitis C-Associated Lymphoproliferative Diseases

As hepatitis C plays an important role in lymphomagenesis, it is postulated that eradication of virus may produce a response in its haematological manifestation as well. In a review of eighteen hepatitis C infected patients with indolent B-cell lymphoma, an overall response rate of the lymphoma after antiviral treatment alone was 63% and 80%, respectively, in the group receiving interferon plus ribavirin and pegylated interferon plus ribavirin, respectively [28]. There was, however, persistent presence of cryoglobulin in majority of the cases despite the absence of detectable tumour. In another study of nine hepatitis C infected patients with splenic marginal zone lymphoma, seven of them had sustained virological response after antiviral treatment (which consisted of interferon as first line therapy with ribavirin added if unsatisfactory response). All seven patients had a concomitant haematological response with a decrease in splenic size and disappearance of villous lymphocytes from peripheral blood [28]. In the remaining two patients who had persistent detectable hepatitis C RNA in blood, there was no significant clinical haematological response. The immunoglobulin gene rearrangement observed at diagnosis in these patients was still detectable in patients achieving a complete clinical remission. Although antiviral treatment has a therapeutic role in the treatment of hepatitis C-associated lymphoproliferative diseases, most of the patients still have detectable residual diseases by molecular methods. Antiviral therapy alone is unable to achieve a complete remission of their haematological diseases in these patients.

9. Conclusion

There are different opinions on the risk of hepatic dysfunction during chemotherapy for haematological patients with chronic hepatitis C infection. No consensus guidelines concerning the management of these patients are currently available and more studies on this issue are warranted.

The role of hepatitis C infection in lymphomagenesis is intriguing and a better understanding may help our management of hepatitis C-associated lymphoproliferative diseases. Although antiviral treatment may not be able to eradiate hepatitis C-associated lymphoma, it should be considered as a treatment option for tumour control, especially for those who may not be able to tolerate cytotoxic chemotherapy.

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Hindawi Publishing Corporation
Hepatitis Research and Treatment
Volume 2010, Article ID 267412, 6 pages
doi:10.1155/2010/267412

Research Article

Treatment of Chronic Hepatitis C Virus Infection in Dialysis Patients: An Update

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Received 27 May 2010; Revised 19 August 2010; Accepted 24 August 2010

Academic Editor: Tatehiro Kagawa

Hepatitis C virus (HCV) infection is a blood-borne infection and its prevalence used to be elevated in hemodialysis (HD) patients. Its main mode of contamination relies on nosocomial transmission. HCV infection is frequently associated in HD patients with normal liver enzymes whereas liver histology can display some degree of HCV-related lesions. The assessment of HCV-related lesions, even in HD dialysis patients, can be done via noninvasive tests. After kidney transplantation, HCV-related lesions can worsen; however, in this setting antiviral treatment harbors the risk of acute rejection. Therefore, it is recommended to implement antiviral treatment while the patient is receiving dialysis therapy. In this setting, the rate of viral clearance is usually high. In case of sustained virological response, no relapse occurs after kidney transplantation, despite heavy immunosuppression.

1. Introduction

The most important forms of liver disease in dialysis patients are viral hepatitis B (HBV) and C (HCV). The vast majority of literature on dialysis for hepatitis refers to hemodialysis (HD). Individuals receiving peritoneal dialysis (PD) are at less risk of acquiring blood-borne infections for several reasons, including an absence of extracorporeal blood manipulation, a lack of intravascular access, as well as a lower requirement for blood transfusions. Also, PD takes place in the patient's home, where there is no exposure to other patients.

An accurate assessment of the natural history of HCV in dialysis patients is not easy to obtain. HCV infection in dialysis patients is often asymptomatic with an apparent indolent course. HCV infection extends over decades rather than years whereas chronic kidney-disease (CKD) patients generally have higher morbidity and mortality rates than those of the general population, due to age and comorbidity conditions [1]. This makes the long-term consequences of HCV difficult to establish. Additional factors also modify the course of liver disease, including HBV/HCV coinfection, coinfection with human immunodeficiency virus, or alcohol abuse.

Because of the wide use of antiviral drugs and because posttransfusional hepatitis no longer occurs, future natural-history studies on chronic HCV will become less possible [2]. Accurate evaluation of HCV infection in the CKD population is further complicated by the observation that aminotransferase values are typically lower in dialysis than nonuremic populations [3]. However, dialysis patients that do show detectable HCV RNA have aminotransferase levels

greater than those who do not, although values are typically within the "normal" range [4, 5]. Therefore, if one wants to assess the impact of chronic HCV infection in CKD patients, a liver biopsy is usually performed [2]. However, a liver biopsy may be replaced by noninvasive tests, such as a FibroTest or a FibroScan [6, 7], and these tests are of particular interest when there is a possibility of kidney transplantation to treat CKD.

2. HCV-Related Outcomes in the CKD Population

A recent meta-analysis on the impact of HCV on mortality in 11,589 maintenance-dialysis patients, from seven observational studies, concluded that the estimated adjusted relative risk (aRR: all cause mortality) was 1.34 (1.13–1.59; $P < .01$) [8]. The cause of death as hepatocellular carcinoma and the incidence of liver cirrhosis, were significantly more frequent among anti-HCV-positive than anti-HCV-negative dialysis patients in all seven studies. The unadjusted summary estimate for liver-related mortality was 5.89 (1.93–17.99; $P < .001$) according to a random-effects model [8].

Recently, Kalantar-Zadeh et al. [9] evaluated a database of 13,664 chronic HD patients in the United States who had undergone HCV serology. They observed that the mortality-hazard ratio was strongly associated with HCV infection: that is, it was 1.25 (1.12–1.39; $P < .001$). Thus, when HCV-positive CKD patients undergo kidney transplantation, it is possible that the natural course of chronic HCV infection is altered by the use of chronic immunosuppression. Indeed, two studies have shown that survival was significantly improved in HCV-positive patients who had benefited from a kidney transplant compared to those who remained on a kidney waiting list [10, 11].

After kidney transplantation, within the first 5 years post-transplant, patient survival is similar in both HCV-positive and HCV-negative patients [12–14]. However, when 10-year survival rates are examined, HCV then appears to be a detrimental effect [12–14]. A meta-analysis of observational studies identified eight clinical trials (6,365 unique patients) in which the presence of anti-HCV antibodies in the serum was an independent and significant risk factor for death and graft failure after kidney transplantation. The estimates for relative risk (RR) were 1.79 (1.57–2.03) and 1.56 (1.35–1.80), respectively [15].

The adverse impact of HCV on survival after kidney transplantation has been linked to liver dysfunction. Furthermore, a positive anti-HCV serology in kidney-transplant patients has been implicated in the development of *de novo* glomerulopathy [19], an increased incidence of serious infections [20], and new-onset diabetes mellitus [21]. In addition, in kidney-transplant patients, the use of alpha-interferon (αIFN) to treat HCV infection has been associated with (i) a poor response to antiviral therapy and (ii) the occurrence of a high rate of acute rejection, that is, up to 50% in some series [22, 23]. The latter were mainly humoral (sub)acute rejections [24]. Conversely, in kidney-transplant patients, the use of pegylated alpha-interferon (pegαIFN), although more limited, has been rarely associated with acute allograft rejection [25]. Because of the above concerns, it seems reasonable to treat HCV infection while the patient is on dialysis, that is, before they are placed on a kidney-transplant waiting list.

3. HCV Treatment in the General Population

At the moment, the best treatment for chronic HCV infection in patients with normal renal function relies on the combined use of pegαIFN and ribavirin (RBV). Thus, Hartwell and Shepherd recently performed a meta-analysis that included ten randomized, controlled trials (RCTs) in which treatment was based on pegαIFN/RBV or αIFN/RBV [26]. pegαIFN/RBV therapy resulted in significantly higher sustained virological response (SVR) rates than treatment with the combined αIFN/RBV therapy. Treatment for 48 weeks with pegαIFN/RBV was significantly more effective than the same treatment for 24 weeks. Significantly higher SVR rates were seen with combined αIFN/RBV compared to either an αIFN monotherapy or to no treatment. In this meta-analysis (four αIFN trials), the relative risk of not experiencing an SVR was 0.59 (95% CI, 0.51–0.69) and was highly statistically significant ($P < .00001$). SVRs were higher for patients with genotype non-1 compared with genotype 1 for both pegαIFN/RBV and IFN/RBV treatments [26].

4. HCV Treatment in the CKD Population

The AASLD (American Association for the Study of Liver Diseases) has published guidelines for the CKD population stating that "when HCV infection is identified in persons with CKD, interferon-based antiviral treatment must be considered, but the regimen will vary depending of the kidney disease. . .The decision to treat must take into account the competing severities of the CKD and the chronic liver disease, the risks of the treatment itself, . . ., and whether there are comorbid conditions that may affect morbidity and mortality, such as cardiovascular disease." [27].

The kidneys play a major role in the catabolism and filtration of both interferon and ribavirin; thus, their clearances may be affected in subjects with CKD [28, 29]. The clearance of pegylated interferon is affected in those with CKD, although hemodialysis does not affect its clearance [30]. Hence, the AASLD guidelines recommend subcutaneous weekly doses of 1 μg/kg of peginterferon alpha-2b and of 135 μg of peginterferon alpha-2a [27] to patients with stage 3–5 CKD. Because ribavirin is eliminated by the kidney, and if overdosed might result in dramatic anemia [31], ribavirin therapy is contraindicated when creatinine clearance is <50 mL/min. Hence, most data regarding HCV treatment in the CKD population deal with the use of either standard α-interferon or α-pegylated interferon.

5. Treatment of Chronic HCV Infection in CKD Patients

With regards to end-stage kidney-disease (ESKD) patients who are chronically treated by dialysis, Casanovas-Taltavull

Table 1: Treatment with alpha-interferon or pegylated-alpha interferon in dialysis HCV positive patients: results from 3 meta-analyses.

	Meta-analysis 1 (Fabrizi et al. [16])	Meta-analysis 2 (Gordon et al. [17])	Meta-analysis 3 (Alavian and Tabatabaei; [18])
Number of studies	28	25	33
Number of patients	645	459	770
Overall SVR (standard IFN/Peg-INF) %	39/41	41/37	39.1/39.3
Genotype 1(%)	33	Not reported	Not reported
Treatment discontinuation (standard IFN/Peg-IFN/Placebo) %	19/27/ not reported	26/28/22	22.6/29.7/not reported

SVR: sustained virological response; IFN: alpha-interferon; Peg-IFN: pegylated alpha-interferon.

et al. reviewed two meta-analyses (Meta-1 and Meta-2) published in 2008 (Table 1). From these, they analyzed the SVRs, any adverse effects, and the reasons for discontinuing αIFN treatment in dialysis patients [32]. The Meta-1 study analyzed results obtained from 645 patients; the Meta-2 study used data from 459 patients (19 studies were duplicated). Overall, the SVR was 40%; SVR in genotype 1 was 33%, with pegylated interferon providing few additional benefits over conventional alpha-interferon. Adverse events, such as typical flu-like syndrome occurred in 41% of patients, requiring withdrawal of antiviral treatment in 11% of them. A high rate of anemia was also documented, although the use of recombinant erythropoietin, intravenous iron administration, or transfusions was not generally reported. A typical flu-like syndrome occurred in 41% of patients, which required withdrawal of antiviral treatment in 11%. Severe adverse events were divided into the following groups: hormonal (thyroid), bone pain, cytopenia, gastrointestinal, immunological (prior graft rejection), central nervous system, cardiovascular, and infectious problems. The reviewers of these meta-analyses pointed out any bias in the selection criteria of candidates for treatment, limitations related to the number and type of adverse effects (as well as their clinical evaluation), and discrepancies in cases of discontinuation of treatment or loss to follow-up.

With regards to Meta-1, the primary outcome was a SVR (as a measure of efficacy); the secondary outcome was the drop-out rate (as a measure of tolerability) [16]. They identified 13 prospective studies, which were controlled clinical trials that included 539 unique patients, of whom 10 (76.9%) patients were receiving maintenance dialysis. Pooling of these studies' results showed a significant increase in viral response of patients treated with antiviral therapy compared to patients who did not receive any therapy (controls). The pooled odds ratio (OR) of failure to obtain a SVR was 0.081 (95% confidence intervals (CI), 0.029–0.230), $P = .0001$. The pooled OR of drop-out rate was significantly increased in treated versus control patients, OR = 0.389 (95% CI, 0.155–0.957), $P = .04$. The studies were heterogeneous with regard to viral response and drop-out rate. In the subset of clinical trials ($n = 6$) involving only dialysis patients receiving α-IFN monotherapy for chronic HCV, there was a significant difference in the risk of failure to obtain a SVR (study versus control patients), OR = 0.054 (95% CI, 0.019; 0.150), $P = .0001$. No difference in the drop-out rate between study and control patients was shown (OR = 0.920 (95% CI, 0.367; 2.311), NS). Meta-1 showed that viral response was greater in patients with chronic kidney disease who received antiviral therapy than in controls. No differences in the drop-out rates between study and control patients occurred in the subgroup of dialysis patients on α-IFN monotherapy [16].

With regards to Meta-2, the authors took into account those chronic dialysis patients with chronic HCV infection who were either treated with αIFN or pegαIFN, with or without ribavirin [17]. They searched on MEDLINE for indexed studies since 1966, and only selected studies with a sample size greater than 10. They looked for the following parameters: SVR at 6 months after treatment, rate of treatment discontinuation caused by adverse events, and factors associated with these outcomes. They analyzed 20 studies that contained 459 αIFN-treated patients, three studies that contained 38 pegαIFN-treated patients, and two studies that contained 49 pegαIFN and ribavirin-treated patients. The overall SVR rate was 41% (95% confidence interval [CI], 33 to 49) for αIFN and 37% (95% CI, 9 to 77) for pegαIFN. Treatment-discontinuation rates were 26% (95% CI, 20 to 34) for αIFN and 28% (95% CI, 12 to 53) for pegαIFN. SVR was higher, with 3 million units (MU) or higher of αIFN at three times weekly, with lower mean amounts of HCV RNA, lower rates of cirrhosis, a HCV genotype 1, or elevated transaminase, though these findings were not statistically significant.

Treatment-discontinuation rates were greater in studies using larger doses. Hence, side-effects from alpha-interferon were numerous, particularly in the ESKD population. The main side effects were fatigue/weakness and loss of appetite, which may lead to weight loss and, thus, fluid overload if dry weight is not adapted accordingly. Many patients also developed anemia, which often requires commencement or increased treatment with erythropoietin-stimulating agents; in addition, seizures can occur if there is fluid overload and hypertension. The limitations of these meta-analyses were publication bias, there were few randomized controlled trials, and there were limitations in generalizability of all hemodialysis patients. In conclusion, alpha-IFN treatment of hemodialysis patients resulted in an SVR rate of 41%. Thus, a higher weekly dose of αIFN, a lower mean level of pretreatment HCV RNA, a lower rate of cirrhosis, an HCV genotype different from 1, and/or decreased transaminase levels may all be associated with greater SVR rates [17].

A more recent meta-analysis has been published on a group of 770 hemodialysis patients with chronic HCV infection (Table 1), in which the authors evaluated factors that were associated to SVR after α-pegylated or standard α-IFN monotherapy. Twenty-one studies on α-IFN-alfa2a or α-IFN-alfa2b (491 patients) and 12 on pegylated-IFN-alfa2a or PEG-IFN-alfa2b (279 patients) were evaluated. The pooled SVRs for standard and pegylated α-IFN monotherapy in random-effect models were 39.1% (95% CI, 32.1 to 46.1) and 39.3% (95% CI, 26.5 to 52.1), respectively. Pooled dropout rates were 22.6% (95% CI, 10.4 to 34.8) and 29.7% (95% CI, 21.7 to 37.7), respectively. Female gender, HCV-RNA copies per mL, HCV genotype, alanine transaminase pattern, duration of infection, stage of liver fibrosis, and treatment duration were not associated with SVR. Only an age less than 40 years was significantly associated with SVR (odds ratio, 2.17; 95% CI, 1.03 to 4.50) [18].

There are only few limited reports that describe the combined use of (peg)alpha-interferon and ribavirin in dialysis patients. With regard to this combined therapy, the AASLD guidelines state that "Ribavirin can be used in combination with interferon with a markedly reduced daily dose with careful monitoring for anemia and other adverse effects." [27]. The largest series published so far on the combined use of peginterferon alpha-2a plus ribavirin in hemodialysis patients obtained a SVR rate of 97% (34/35) in the treated patients (peginterferon alpha-2a plus ribavirin) versus 0% (0/35) in untreated controls [33]. These findings have not been confirmed in further reports where the SVR rate ranges between 7% and 71% [1].

6. Treatment of Acute HCV Infection in CKD Patients

In the general population, with regard to the treatment of acute HCV infection, the AASLD guidelines state that "Treatment can be delayed for 8 to 12 weeks after acute onset of hepatitis to allow spontaneous resolution; ...Although excellent results were achieved using standard interferon monotherapy, it is appropriate to consider the use of peginterferon...Until more information becomes available, no definitive recommendation can be made about the optimal duration needed for treatment of acute hepatitis C; however, it is reasonable to treat for at least 12 weeks and 24 weeks may be considered." [27].

In dialysis patients, Liu et al. have very recently published their experience regarding the treatment of acute HCV infection. They included 35 dialysis patients that had no spontaneous clearance of HCV at 16 weeks after acute HCV infection. They were thus then given a course of peginterferon alpha 2a at 135 μg weekly for 24 weeks [34]. They compared the results with those from a historical series of 36 hemodialysis patients who had acute hepatitis C, but had not received treatment. The rate of SVR in their treatment group was significantly higher than the rate of spontaneous HCV clearance in the control historical series group (88.6% versus 16.7%). All but one patient had a rapid virologic response (undetectable HCV RNA levels at 4 weeks of therapy), and all patients who received more than 12 weeks of therapy had early and end-of-treatment virologic responses. All patients who had clearance of HCV by 16 weeks had undetectable HCV RNA levels during and at the end of follow-up. Liu et al. conclude that "Pegylated IFN alfa-2a monotherapy is safe and efficacious for hemodialysis patients with acute hepatitis C. It is suggested that patients without spontaneous clearance of HCV by week 16 should receive this therapy." [34].

In addition, dialysis patients who were cleared of the HCV virus after antiviral therapy, and received kidney transplantation, did not present with HCV reactivation, despite heavy immunosuppression [35]. Hence, 16 HCV seropositive/HCV RNA-positive hemodialysis patients who were treated with IFN-alpha (9 MU/wk during 6 or 12 months) underwent kidney transplantation 38 months (range: 2 to 57) after alpha-IFN therapy. At kidney transplantation, HCV viremia was negative in all patients. Immunosuppression relied on anticalcineurin agents with or without steroids and/or antimetabolites; in addition, 12 of them received induction therapy with antithymocyte globulins; at the last follow-up after kidney transplantation, that is, 22.5 months (range, 2 to 88), HCV viremia remained negative in all patients [35]. Recently, we have assessed the persistence of HCV infection in 26 HCV seropositive kidney-transplant patients currently receiving immunosuppressants, and who were formerly infected with HCV, that is, they had eliminated HCV either spontaneously or after interferon-α therapy while on hemodialysis [36]. No biochemical or virological relapse was seen during the median posttransplant follow-up of 10.5 years (range: 2–16) in those patients who received immunosuppressive therapy that included calcineurin inhibitors (100%), and/or steroids (62%), and/or antimetabolites (94%). At the last follow-up, all had undetectable HCV RNA according to the conventional tests that were repeated, on average, five times (range, 1–15). We also looked for residual HCV RNA in their plasma and peripheral blood-mononuclear cells (PBMCs) (stimulated or not in culture) with an ultrasensitive RT-PCR assay, followed by Southern blotting for PBMCs: no HCV genomic RNA was detected in the plasma samples or in the unstimulated and stimulated PBMCs. Thus, an absence of a relapse of HCV in formerly HCV-infected immunocompromised patients suggests complete eradication of HCV after its elimination while on dialysis [36]. These findings highlight the fact that HCV-positive dialysis patients who have a SVR after completion of alpha-(peg)interferon therapy are really cured of HCV.

We conclude that, because it is not always safe to treat HCV infection after kidney transplantation, antiviral treatment should be implemented before transplantation, that is, while the patient is on dialysis therapy. The evidence suggests that treatment might be based on alpha interferon (standard or pegylated), this results in a high rate of sustained viral clearance. In cases where there is no virological response, one could add very low doses of ribavirin therapy to the alpha-interferon in order to maximise the virological response. However, one needs to be mindful of the risk of hemolytic anemia. Finally, dialysis HCV seropositive patients who have a sustained virological response after antiviral therapy do

not relapse after kidney transplantation despite powerful immunosuppressive therapy.

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Hindawi Publishing Corporation
Hepatitis Research and Treatment
Volume 2010, Article ID 475746, 9 pages
doi:10.1155/2010/475746

Review Article

Antiviral Treatment for Hepatitis C Virus Infection after Liver Transplantation

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Received 21 May 2010; Revised 13 August 2010; Accepted 6 October 2010

Academic Editor: Tatehiro Kagawa

A significant proportion of patients with chronic hepatitis C virus (HCV) infection develop liver cirrhosis and complications of end-stage liver disease over two to three decades and require liver transplantation, however , reinfection is common and leads to further adverse events under immunosuppression. Pretransplant antiviral or preemptive therapy is limited to mildly decompensated patients due to poor tolerance. The mainstay of management represents directed antiviral therapy after evidence of recurrence of chronic hepatitis C. Combined pegylated interferon and ribavirin therapy is the current standard treatment with sustained viral response rates of 25% to 45%. The rate is lower than that in the immunocompetent population, partly due to the high prevalence of intolerability. To date, there is no general consensus regarding the antiviral treatment modality, timing, or dosing for HCV in patients with advanced liver disease and after liver transplantation. New anti-HCV drugs to delay disease progression or to enhance viral clearance are necessary.

1. Introduction

According to the World Health Organization, 130 to 170 million people are chronically infected with hepatitis C virus (HCV) and 3 to 4 million people are newly infected each year worldwide [1]. The median time to develop cirrhosis is estimated to be 30 years, and 33% of patients have an expected median time to the development of cirrhosis of less than 20 years. Although antiviral therapy is available, the number of patients with end-stage liver disease due to HCV will continue rise over the next 10 years [2]. HCV is the major cause of chronic liver disease, cirrhosis, and liver cancer in most developed countries [3], including Japan [4]. It is the most common indication for liver transplantation in developed nations [5, 6].

Liver transplantation is an effective treatment to reduce morbidity and mortality in this population. Reinfection with HCV, however, is a critical complication with major effects on graft and patient survival. Indeed, the real challenge of controlling HCV begins after liver transplantation under life-long immunosuppression, and mitigating the damage is a crucial concern. In this paper, we focus on this challenging aspect, evaluating the available treatment options and strategies against HCV before and after transplantation to prevent reinfection with the goal of eradicating recurrent infection.

2. Clinical Course after Liver Transplantation

Spontaneous clearance of HCV after transplantation is rare [7–11], and reinfection of the allograft is common [12, 13]. Histologic progression of HCV under immunosuppressive therapy is more rapid than that in nontransplant patients [14, 15]. HCV patients have a poorer prognosis after liver transplantation than those with other indications [16–19], a finding that remains unchanged in recent research [20]. Large studies have demonstrated that recipients with HCV have approximately 10% lower 5-year graft and patient survival rates than non-HCV controls [18, 19]. One study comparing approximately 7500 HCV recipients and 20,000 non-HCV recipients reported an overall 3-year patient survival rate of 79% in HCV patients and 81% in non-HCV patients [16]. Factors with a significant negative impact

on patient survival include a preoperative model for end-stage liver disease (MELD) score [21], fibrosis stage of 2 or greater at 12-month biopsy, advanced donor age, history of hepatocellular carcinoma (HCC), and early acute rejection [22].

HCV reinfection occurs during transplantation in the reperfusion phase of the graft, and acute hepatitis can usually be detected 1 to 3 months after transplantation [23]. The clinical course following reinfection varies. In general, 8% to 30% of the recipients will present with severe progressive disease within 5 years [15, 17, 24]. The median time to cirrhosis in the nontransplant patients was 30 years [25] and in the transplanted patients with HCV disease is expected to be 10 years. The risk of decompensation is 50% within 1 year after diagnosis in the absence of therapeutic intervention [15, 26]. A small percentage of recipients can develop an early cholestatic hepatitis within the first year after transplantation with a risk (2%–8%) of progressive liver dysfunction and rapid development of cirrhosis [12, 27]. Retransplantation in these patients is associated with poor outcomes and is controversial [28].

3. Risk Factors for Severe Recurrent HCV

The reported risk factors include advanced donor age [29, 30], high viral load in the preoperative or early postoperative periods [31, 32], treatment of acute rejection [28], long duration between the antiviral therapy and transplantation [16, 18, 33], and baseline pre transplant liver function [34]. The expression of cytokeratin 19 and that of vimentin in liver biopsies without fibrotic changes (F0) [35] are also considered risk factors for severe recurrent HCV. Postoperative insulin resistance diabetes mellitus [36, 37], metabolic syndrome [38], and lipid peroxidation (oxidative degradation of lipids) [39] are reported to be associated with severe HCV recurrence.

The role of corticosteroids in severe recurrent HCV is complicated. Steroid bolus injection as acute rejection therapy is one of the risk factors for severe HCV recurrence and graft loss [19]. Rapid withdrawal of corticosteroids might cause graft fibrosis [40], but a corticoid-free regimen may be promising [41]. A meta-analysis of randomized trials using a corticoid-free immunosuppression regimen showed a significant reduction of HCV recurrence in corticoid-free groups [42].

The impact of other immunosuppressive agents, including mycophenolate mofetil, azathioprine [43], or interleukin-2 inhibitors [44] on severe recurrent HCV remains controversial. OKT3 is associated with increased graft loss and increased mortality [12].

A viral quasispecies is a group of viruses related by a similar mutation or mutations, competing within a highly mutagenic environment. After transplantation, diversification of hypervariable region 1 is decreased, and the virus population becomes more homologous [45] although the changes might be temporary [46]. A more complex HCV hypervariable region 1 quasispecies population was reported to be associated with HCV recurrence after transplantation [47, 48].

4. Viral Kinetics

Powers et al. [49] confirmed that viral loads begin to rise 15 hours after the anhepatic phase. In total, 19% hepatocytes are infected in an average of 37 days (range: 4–82 days) after transplantation. Schiano et al. [50] demonstrated accelerated HCV kinetics in LDLT recipients ($n = 11$) compared to deceased donor liver transplantation (DDLT) recipients ($n = 15$). In their study, HCV RNA levels rose more rapidly in LDLT patients; the differences in patient and graft survival, however, did not reach statistical significance.

Another study [51] focusing on the histologic aspects of HCV recurrence with protocol biopsy reported more severe progression of HCV disease in LDLT compared to DDLT. Other studies [52, 53] have also demonstrated that individuals with a high level of replication in the perioperative period develop more fibrosis in the allograft 1 year after transplantation. When the graft develops fibrotic or cirrhotic changes, the patient prognosis is dismal.

5. Pretransplant Antiviral Therapy

Antiviral treatment of patients with mildly decompensated cirrhosis (model for end-stage liver disease, MELD > 18) is a more problematic approach than treatment after transplantation.

Suitable candidates for pretransplant therapy would be treatment-naïve patients or prior relapsers to standard interferon (IFN) and ribavirin (RBV) treatment because the chances for an on-treatment virologic response before transplantation are high [27]. Patients with predictable timing of transplantation such as those with living donors or those with HCC may be good candidates for pre-transplant therapy. The use of growth factors is considered an option to treat therapy-associated anemia and leucopenia as it can improve quality of life and may decrease the need for antiviral therapy dose reduction [54].

Everson et al. [55] reported his experience in the treatment of 102 patients with chronic hepatitis and decompensated liver disease using a low accelerating dosage regimen of IFN alfa-2b plus RBV. Serum HCV RNA was cleared in approximately 40% of patients on this treatment, and 22% achieved a sustained virologic response (SVR).

Forns et al. [56] administered antiviral therapy to patients with IFN alpha-2b 3 MU/day and RBV 800 mg/day when the expected time until transplantation was less than 4 months. Of 30 patients enrolled, 9 (30%) achieved a virologic response and 21 did not respond to therapy. Of the nine, six remained free of infection after a median followup of 46 weeks and HCV infection recurred in three patients after transplantation. In contrast, Smallwood et al. [57] reported that patients treated with IFN before transplantation have a significantly earlier and more aggressive recurrence of HCV. Carrión et al. [58] performed a case control study comparing 51 patients who underwent treatment with IFN and RBV and a control group (untreated 51 individuals awaiting transplantation who were matched by age, Child-Pugh, and time on the waiting list). There was a higher incidence of bacterial infections after transplantation in treated patients,

particularly in Child-Pugh B-C individuals. Further data are needed before a definitive conclusion can be drawn.

6. Early Posttransplant Antiviral (Preemptive) Therapy

6.1. Its Rationale and Use in LDLT Patients. Preemptive treatment may be beneficial in potentially tolerant patients, such as patients with a lower natural MELD score undergoing liver transplantation for HCC within the Milan criteria, with an outcome comparable to that of non-HCC cases, and also in well-planned LDLT cases [59] with splenectomy [60]. The preemptive approach may provide an oncologic benefit, which has been demonstrated in nontransplant patients with HCV who underwent liver resection for HCC [61].

The rationale for preemptive therapy is to strike at a time when the total HCV viral load is relatively low after liver transplantation [23, 49] and histologic damage is absent or minimal. Delay of histologic damage can be expected [62, 63]. Garcia-Retortillo et al. [23] observed a rapid decrease in the viral load during the anhepatic phase in 20 patients who underwent liver transplantation for HCV. The viral load continued to exponentially decrease after graft reperfusion with a progressive increase after the first week of transplantation, reaching a plateau by the end of the first month.

We examined the feasibility and efficacy of a preemptive combination of IFN and RBV therapy against HCV in LDLT patients [64]. Tolerance was a limiting factor, with 25% of the patients deviating from the planned protocol. Overall, among the 23 patients enrolled, 9 (39%) achieved SVR. The cumulative 3-year survival rate did not differ between the enrolled patients and the HCV-negative patients (90%) during the study period [59]. The results of the study were encouraging and demonstrated an optimal window for treatment initiation and the optimal doses of IFN and RBV necessary for effective viral eradication [65] after LDLT.

6.2. In DDLT. The overall efficacy and feasibility of preemptive therapy in DDLT is controversial. Randomized studies on a preemptive approach with IFN monotherapy [66, 67] or combined therapy [63] demonstrated a reduced or delayed incidence of hepatitis after liver transplantation, but not the prevention of viremia. Discontinuation of the treatment was necessary in 30% of the subjects in the study. Mazzaferro et al. [68] treated 36 recipients with IFN-alpha-2b (3 million IU, 3 times/week) and RBV (10 mg/kg per day). The treatment was initiated at a median of 18 days after the operation and continued for a year. After a median followup of 52 months, 5-year patient survival was 88%. Serum HCV RNA was cleared in 12 patients of the 36 recipients (33%) after a median of 37 days. These patients remained negative for serum HCV RNA for a median of 36 additional months without antiviral treatment. Dose reduction was necessary in 9 (25%) patients. Although these outcomes were encouraging, the current understanding is that the adverse events associated with treatment outweigh the theoretical benefits of preemptive therapy [12].

Shergill et al. [69] reported about the results of preemptive treatment. Only 51 (41%) of 124 transplant recipients were eligible for preemptive treatment; eligible patients had lower MELD and Child-Pugh scores pretransplantation. Dose reductions and discontinuations were required in 85% and 37% of patients, respectively, and 27% experienced serious adverse events. Only 15% of patients were able to achieve full-dose treatment during treatment. End-of-treatment rate and SVR were 14% and 9%, respectively.

7. Treatment for Established Infection

The recommendation of the International Liver Transplantation Society is to perform post-transplant surveillance and protocol biopsies to detect recurrent HCV disease [70] and to start combined pegylated-(peg-) IFN and RBV therapy for stage II fibrosis [12]. Treatment of HCV recurrence is mostly modeled after the strategy of treating HCV in nontransplant patients [5, 6]: Studies of a noncontrolled series of patients [71–81] revealed an efficacy of 26% to 50% after peg-IFN and RBV therapy, a higher efficacy than that of conventional IFN and RBV therapy.

These studies suggested that factors associated with higher probabilities of a viral response were nongenotype 1, low pretreatment HCV RNA levels, absence of advanced cirrhosis, early virologic response ($\geq$ 2 log drop in HCV RNA from baseline at 3 months), and adherence to combination therapy [71, 82–93] similar to that in nontransplant patients. Other reports [90, 94] suggested that patients on cyclosporine had a higher SVR. Berenguer et al. [73] reported a higher SVR rate for nongenotype 1 (60%) versus genotype 1 (31%) and a higher SVR rate with peg-IFN with RBV compared to standard IFN with RBV (50% versus 13%). Combined treatment with peg-IFN is suggested to be effective among those who failed previous conventional IFN and RBV therapy, achieving an SVR in 30% with histologic improvement [95]. These studies were, however, limited by small sample sizes and a lack of randomized controlled trials.

Carrión et al. [96] studied patients with mild HCV recurrence (fibrosis stage F0-2) that were randomized into two groups; group A, no treatment; group B, treated with combined peg-IFN-alpha-2b (1.5 μg/kg/week) and RBV (adjusted for renal function, maximum dose 1200 mg/day) for 48 weeks. Median time to treatment was 14 months. An SVR was achieved in 0 (0%) and 13 (48%), respectively. Histologic stabilization or improvement, which corresponded with improvement in the hepatic venous pressure gradient, was recognized with an SVR. Early virologic response was an independent significant factor predicting SVR in the study, as described previously [97–99]. Dose reduction was necessary in 67% of cases, and interruption was necessary in 56%. An increased risk of rejection in the treatment groups was not substantiated. The study demonstrated the advantage of antiviral treatment with peg-IFN and RBV, which achieves permanent viral clearance in a high proportion of patients when initiated at the stage of mild HCV recurrence.

One systematic review [100] of the efficacy of post-transplant treatment with standard IFN (IFN-RBV) [93, 101–117] or peg-IFN in combination with RBV (peg-RBV)

[77–80, 118–120] given for 6 to 12 months included 38 studies. Patients were predominantly men with a high rate of genotype 1 infection (> 80%). IFN-RBV was associated with an end-of-treatment virologic response rate of 34% and an SVR rate of 24%, while peg-RBV was associated with an end-of-treatment virologic response rate of 42% and an SVR rate of 27%. Pooled discontinuation rates were 24% with IFN-RBV and 26% with peg-RBV. Only 33% with IFN-RBV and 21% with peg-RBV completed the intended protocol. The majority of patients required dose reduction. The overall rate of acute graft rejection was 2% with IFN-RBV and 5% with peg-RBV. The authors concluded that combination therapies have similar tolerability and safety, but the advantage of peg-RBV in terms of viral response remains unclear, and further studies are required.

Another systematic review [121] focused only on studies using peg-IFN in combination with RBV as post-transplant treatment. The 19 reviewed studies showed a mean end-of-treatment response of 42% and a mean overall SVR of 30%. A recent study [122] disclosed an SVR rate of 64% in DDLT patients. The antiviral treatment regimen comprised pegylated-IFN (180 μg) every 2 weeks and RBV at a dose of 200 to 400 mg every day. The treatment duration was flexible and individualized, and it depended on the viral response to treatment. The dosage of tacrolimus was decreased gradually.

8. Nonresponders

When an SVR cannot be achieved, the next possible best step is to prevent the progression of fibrosis. Peg-IFN may be histologically beneficial even when HCV eradication is not obtained [123, 124]. Studies of nonresponders to determine the optimal dosage and duration of either IFN, RBV, or both are lacking in terms of delayed disease progression. Administration of oral ursodeoxycholic acid may be beneficial in terms of improving the biochemical response, but not histologic changes, as recently demonstrated in a large-scale, multicenter, double-blind trial in a nontransplant population [125]. It has no clear benefits in liver transplant recipients [126].

In a subset of patients with a progressive cholestatic variant of HCV disease [127] despite combined IFN and RBV therapy, indefinite continuation of antiviral therapy [128], or temporary treatment with double-filtration plasmapheresis [129, 130], a novel option to decrease HCV viral load, in combination with antiviral therapy may be effective [130]. Further studies focusing on the long-term risks and benefits of various treatment modalities for patients nonresponsive to antiviral therapy are necessary.

9. Future Perspectives

Previous descriptions [131] as well as the descriptions above indicate the need for additional treatment modalities. The treatment goal should be eventual viral eradication. In theory, this goal can be achieved by disrupting the steady-state HCV kinetics by reducing virion production, thereby allowing infected cells to be eliminated [132, 133]. Lang [134] classifies the future drugs currently under investigation into four categories: new IFNs, RBV alternatives, specific HCV inhibitors, and immunomodulators. Some of these drugs are in phase III trials awaiting further clinical evaluation.

As tolerability is the primary problem of the current widely applied combined IFN and RBV treatment after liver transplantation, specific HCV life-cycle inhibitors may have a beneficial role. Among the various drugs under investigation, protease inhibitors such as telaprevir and boceprevir appear to be safe and are currently undergoing clinical evaluation. A clinical trial with triple combinations of peg-IFN, RBV, and telaprevir has been performed [135] in patients with chronic HCV. The SVRs of the patients administered telaprevir for 24 and 48 weeks were 61% and 67%, respectively. High SVR was obtained in patients who had not had a sustained response to the therapy with peg-IFN and RBV [136]. The SVR of the control patients (peg-IFN and RBV for 48 weeks) was 41%. A phase-III clinical trial of boceprevir was begun in 2008 [137–140]. None of the new anti-HCV drugs, however, are currently being evaluated in HCV-infected liver transplant recipients.

10. Conclusions

HCV continues to be a major challenge in liver transplantation. Reinfection is common after transplantation, and progression of the disease leads to a dismal outcome. Pretransplant antiviral therapy with at least on-treatment virologic response at the time of transplantation would be desirable, but the tolerability and risk of therapy limits the applicability to patients with compensated or mildly decompensated liver disease (low MELD score). Preemptive treatment is also limited by frequent complications in the early post-transplant phase and a high rate of side effects. The use of combined peg-IFN and RBV therapy has increased the rate of viral clearance, but its intolerability in the majority of patients prevents its general application. The overall risk and benefit of the current strategy over the long term remains to be evaluated. Additional modalities combining new drugs, such as protease inhibitors, should be pursued.

Abbreviations

DDLT: deceased donor liver transplantation
HCC: hepatocellular carcinoma
HCV: hepatitis C virus
INF: interferon
LDLT: living donor liver transplantation
MELD: model for end stage liver disease
Peg-IFN: pegylated interferon
SVR: sustained viral response
RBV: ribavirin.

Conflict of Interest

None declared.

Acknowledgment

The study was supported by a Grant-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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