

Treatment of Chronic Hepatitis C Virus Infection Among Brazilian Haemophiliacs

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Chronic hepatitis C virus (HCV) infection is now the most important cause of liver cirrhosis and hepatocellular carcinoma worldwide. HCV infection prevalence is high among haemophiliacs (39%-98%), who got infected when received inadequately or non-virus-inactivated large-pool clotting factors concentrates before 1992. Current treatment reduces the probability of developing advanced stages of liver disease. The objective of this study was to evaluate efficacy and safety of the treatment with interferon alpha (IFN) and ribavirin in haemophiliacs. From July 2000 to November 2002, 18 patients were treated with IFN, three million units thrice weekly combined with daily oral doses of 1,000 or 1,250 mg of ribavirin for a minimum of 48 weeks. Eleven patients (61%) showed end of treatment virological response, while nine [(50%): 95% CI: 27-73%] showed sustained virological response as defined by undetectable HCV-RNA six months after treatment. All those nine had persistently undetectable HCV-RNA two to four years post-treatment. There was no treatment interruption due to adverse events. Therefore, the rate of sustained virological response was 50%, with good tolerance.

Key-Words: Chronic hepatitis C, haemophilia, interferon alpha, ribavirin therapy.

Infection by hepatitis C virus (HCV) is one of the greatest public health problems worldwide and one of the main causes of chronic liver disease and death due to liver disease. It is estimated that around 170 million people are living with HCV infection worldwide [1,2].

The HCV is an important cause of morbidity and mortality in patients with hereditary coagulopathy who were treated with coagulation clotting factors not inactivated for virus in the period between the seventies and the mid-eighties [3,4]. In Brazil the importation of lyophilised factor VIII and IX concentrates started in the early 1980s in close proximity with the foundation of the haemophilia unit in February, 1982.

National and foreign studies have shown a high seroprevalence of hepatitis C in haemophiliacs, varying from 39 to 98% [5-7]. The genotypes found in Brazil were 1, 2, 3, 4 and 5, with a predominance of the genotype 1, followed by the genotype 2, but there are regional differences [8]. The genotype 1b predominates among those exposed to lyophilised clotting factor concentrate and is a cause of serious and prolonged liver damage. Barbaro et al. observed in patients with genotype 1b infection frequent ultra-structural alterations of the mitochondria, and the depletion of mitochondrial DNA (mtDNA) in these patients that may represent the expression of a greater impairment of the oxidative phosphorylation process than genotypes 2 and 3, and according to these authors, an increased production of free radicals in patients with genotype 1b may influence the progression of the liver disease by enhancement of HCV cytopathic effect [9].

More than 60% of the patients infected with HCV develop chronic infection, with progression to cirrhosis in 20-30% after

20 to 30 years and a further yearly development of hepatocellular carcinoma of 1-7% [10, 11], almost exclusively in the group with cirrhosis. Therapy for chronic hepatitis C was initiated with interferon alpha in the early 1990s, followed by the addition of ribavirin in 1999 and the use of the pegylated interferon associated with ribavirin since 2001, with the objective of improving the therapeutic response in those patients that did not respond or relapsed six months after end of treatment response. There are many published studies about the treatment of chronic hepatitis in haemophiliacs in the medical literature but none in Brazil.

This study was proposed to evaluate the rate of sustained response and tolerance to combined therapy with the association of interferon alpha and ribavirin for chronic hepatitis C infection in haemophilic patients at Hospital Brigadeiro.

Material and Methods

The Hospital Brigadeiro Haemophilia Unit is a public referral service in the state of São Paulo, and it is a tertiary care hospital with 150 beds and an education unit. Giving the follow-up of large group of patients with hereditary coagulopathy at Hospital Brigadeiro infected by the HCV, seroprevalence was reviewed and a group selected for initiating the treatment protocol by the authors. The selection of the patients was a pilot project that included patients that accepted the treatment. Serological tests were carried out for HbsAg, hepatitis C, HIV and HTLV-I/II in all the patients of the unit, a routine procedure. All serological tests were done through enzyme immunoassay: anti-HIV (Abbott), confirmed by Western blot in a small proportion, anti-HCV and HbsAg (Roche) and the anti-HTLV-I/II (Biomérieux).

An individual diary was developed for laboratory and clinical facts, and drug administration registries during and after treatment. A leaflet was also developed with patients' information, concerning the illness, side effects, contraindications and the modes of drugs application. The patients were oriented about the leaflet fulfilment and about the meetings with the social worker, medical and nursing

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professionals before and during follow-up to improve adhesion to treatment. Informed consent was obtained from patients. Treatment criteria were those published by the Brazilian Ministry of Health guideline in June 21, 2000. Eligible patients were previously untreated adults who had HCV infection (anti-HCV positive, detectable HCV-RNA, with persistent alterations of serum aminotransferases, age above 17 years, at least 15 years of follow-up and negative anti-HIV test). Exclusion criteria were clinical contraindications for the administration of the presumed drugs. Eighteen patients were treated in the period of July 2000 to November of 2002. The therapeutic schedule included: interferon alpha-2 in the dose of three million IU subcutaneous three times weekly associated with ribavirin orally at a dose of 1.0 g daily, for patients lighter than 85 Kg and 1.25 g for patients of higher weight for a period of 48 weeks. Pre-evaluation exams included: biochemical tests (liver and kidney), TSH, T4, anti-thyroid antibodies, protein electrophoresis; prothrombin activity, gastrointestinal upper tract endoscopy, ultrasound scanning of upper abdomen and qualitative HCV-RNA. The HCV-RNA was carried out by the technical INNO-LIPA HCV II. Patients were neither submitted to liver biopsy due to relative contraindication nor to genotyping due to its unavailability in the study period. All patients had pre-treatment abnormal biochemical liver tests as well as detectable HCV-RNA.

The following tests were carried out monthly: serum aminotransferases, kidney function, and blood counts. HCV-RNA, TSH and T4 were done quarterly. A response was deemed positive at the end of treatment when serum alanine aminotransferase was normal and HCV-RNA negative. A sustained response was considered when serum alanine aminotransferase was normal and undetectable HCV-RNA remained until six months after treatment. A relapser was characterized by abnormal serum alanine aminotransferase and positive HCV-RNA in the first six months after treatment. A therapeutic failure (non-responder) was considered when serum alanine aminotransferase persisted altered and detectable HCV-RNA remained three months after treatment. Treatment was interrupted when the patient was considered a non-responder. The patients were followed up for one year period after treatment, with biochemical and hematological tests every three months and if any abnormality was found in HCV-RNA determination. All patients that presented a sustained virological response to treatment were called in July 2005 for new blood collection for HCV-RNA determination to verify if they remained still on sustained response. Those who did not respond at the end of treatment or relapsers were genotyped, when the test became available in the public sector in 2003, through the polymerase chain reaction (PCR) assay, Amplicor® version 2.0.

Statistical Analysis

Medians, means and proportions described patients' demographics and clinical data. Treatment response rates were described as proportions and 95% confidence intervals.

Results

In June 2002, the percentages of positive serological tests were: anti-HCV 286/589 (48.5%), anti-HIV 93/588 (15.8%), HbsAg 17/582 (2.9%) and anti HTLV-I/II 6/585 (1%). In anti-HCV seropositive patients: anti-HIV positivity was 87/286 (30.4%), HbsAg was reagent in 15/286 (5.2%) and anti-HTLV-I/II in 4/286 (1.4%). Out of the 286 patients anti-HCV positive, 71 (25%) were submitted to qualitative HCV-RNA test and 50 of which were positive (70%).

Eighteen patients chronically infected by HCV were treated in a period of two years. Sixteen were male (89%), median and mean ages were of 34 years, varying from 15 to 59 years. Thirteen had haemophilia A (72%), two haemophilia B (11%) and three von Willebrand disease (17%). Seventeen patients were HbsAg negative and one was HbsAg positive. There were no clinical signs of cirrhosis. Eleven patients (61%) responded at the end of treatment, two of them relapsed after three and six months, respectively. Of the nine patients [(50%); 95% CI: 27-73%] who presented sustained virological response, all were persistently HCV-RNA negative after two to four years of follow-up. Post-study genotyping was carried out in all eight non-responders and revealed that six had genotype 1 virus, one genotype 2 and another genotype 3. Some demographic, clinical and treatment characteristics are presented in Table 1. Tolerance to treatment was satisfactory and there was no treatment interruption, except by the temporary reduction of the dose of ribavirin. Thrombocytopenia and neutropenia were mild and occurred in 17% of the patients and did not lead to treatment interruption. Influenza-like symptoms were the most frequent: fever in 44%, headache in 22%, myalgia in 17%, followed by psychiatric symptoms such as irritability and depression in 17%, vomits and abdominal pain in 11% (with reduction of the dose of ribavirin), joint bleeding episodes were referred to have increased in 11%. Pegylated interferon alpha was started in one non-responder but, one month after, he presented thrombocytopenia, which led to its interruption. Thrombocytopenia did not normalize after nine months of suspension of the treatment.

Discussion

In this study, 11 out of 18 patients with chronic hepatitis C infection had therapeutic response at the end of the treatment and a sustained virological response rate of 50%. These patients remained HCV-RNA negative after two to four years of post-treatment follow-up. The side effects neither caused treatment interruption nor interferon dose reduction as they did to ribavirin in two patients.

There are many published studies regarding efficacy and safety of the combined therapy for chronic hepatitis C infection.

Fried et al., in a randomized study of 1,121 patients with chronic hepatitis C virus infection compared the use of peginterferon and placebo, peginterferon and ribavirin and interferon alpha-2b and ribavirin. End of treatment response rates were 59, 69 and 52% and sustained virological response occurred in 29, 56 and 44% of the patients, respectively. The response rate

Table 1. Demographic and clinical characteristics of treated patients

| Patient | Age | Coagulopathy period | Treatment | Genotype virological response | Sustained in responders | HCV RNA in 2005 |
|---------|-----|---------------------|------------------|-------------------------------|-------------------------|--------------------------|
| 1 | 36 | HB | Dec/00- Oct/01 | | Yes | Undetectable |
| 2 | 23 | HA | Nov/01-Oct/02 | | Yes | Undetectable |
| 3 | 53 | vW | Jan/02- Dec/02 | | Yes | Undetectable |
| 4 | 35 | HA | Jul/00 - Jun/01 | | Yes | Undetectable |
| 5 | 44 | HA | Mar/01- Jan/02 | 1 | No | |
| 6 | 19 | HA | Oct/00 - Feb/02 | | Yes | Undetectable |
| 7 | 26 | HA | Oct/00 – Feb/01 | | No | |
| 8 | 46 | HA | Oct/00 – Feb/01 | 1b | No | |
| 9 | 15 | HA | Apr/01- May/02 | | Yes | Undetectable |
| 10 | 52 | HB | Sept/02 – Dec/03 | 1 | No* | |
| 11 | 24 | vW | May/01 – Jul/02 | 1b | No | |
| 12 | 48 | HA | Dec/01- Nov/02 | | Yes | Undetectable |
| 13 | 23 | HA | Dec/01- Nov/02 | | Yes | Undetectable in Apr/2004 |
| 14 | 25 | vW | May/02 – Apr/03 | 1a | No | |
| 15 | 37 | HA | May/01- Apr/02 | 1b | No | |
| 16 | 29 | HA | Jun/01- Apr/02 | 1a | No* | |
| 17 | 23 | HA | Jan/01- Jul/02 | 3a | No | |
| 18 | 44 | HA | Aug/00- Aug/01 | | Yes | Undetectable |

HA: haemophilia A; HB: haemophilia B; vW: von Willebrand disease; No*: end of treatment response and relapse in 3 to 6 months.

to treatment was lower for genotype 1 as compared to genotypes 2 and 3, being 45, 76 and 61% and 21, 46 and 36% for sustained virological response, respectively [12].

Manns et al., in a randomized open-label trial of 1,530 patients with chronic hepatitis C, have assigned interferon alpha-2b plus ribavirin 1,000-1,200 mg/day, peginterferon alpha-2b 1.5 µg/Kg each week plus 800 mg/day ribavirin, or peginterferon alpha-2b 1.5 µg/Kg per week for four weeks then 0.5 µg/Kg per week plus ribavirin 1,000-1,200 mg/day for 48 weeks. The sustained virological response rate was significantly higher in the higher-dose peginterferon group (274/511 [54%]) than in the lower-dose peginterferon (244/514 [47%]) or interferon (235/505 [47%]) groups. Among patients with genotype 1 HCV infection, the corresponding sustained virological response rates were 42% (145/348), 34% (118/349), and 33% (114/343). The response rate for patients with genotype 2 and 3 infections was about 80% for all treatment groups [2].

A Brazilian study by Alves et al., with 400 patients, reported a 33% sustained response rate to treatment with interferon alpha and ribavirin and it was higher in women and in non-genotype-1 virus [13].

There are many observational published studies regarding efficacy and safety of the combined therapy of interferon and ribavirin in haemophilic patients [14].

An Italian study reported 34 haemophilic patients that did not respond to monotherapy with interferon, despite having been treated with higher initial doses, five million UI of interferon alpha for six months followed by three million UI, associated with ribavirin. A sustained virological response to treatment occurred in 37% of the patients, but was lower for

genotype 1 (12%) and 43% in the genotypes 2 and 3. Dose reductions were necessary in 26% of the patients due to side effects. The authors concluded that combined therapy overcomes therapeutic failure to monotherapy in one-third of the patients [15,16]. Lower efficacy of monotherapy with interferon in haemophiliacs had already been documented in a previous study [17].

Another study of Shields et al. in patients with hereditary bleeding disorders observed that 21 out of 28 patients had liver biopsy-confirmed chronic hepatitis. Virological response rate to therapy at three months was 82% (23 out of 28). Three HIV co-infected patients showed an early virological response with loss of HCV-RNA, but two subsequently relapsed three and six months after therapy. Four patients stopped treatment early, because of treatment-related side effects, although three of these have maintained a virological response. Seventeen patients completed the 48-week course. Twenty of the 28 (71%) treated patients have had a durable virological response with a median follow-up of 16 months [18-20].

In the study by Fried et al., it was verified that the sustained virologic response in the combination of interferon alpha-2b and ribavirin in adults and adolescents with inherited coagulation disorders was 29% (16 of 56) compared with 7% (4 of 57) for those started on interferon alone. Among patients younger than 18 years of age who were treated with combination therapy, ten out of 17 (59%) had sustained response compared with six out of 39 (15%) adult patients on the same regimen. In conclusion, in HCV infection patients with inherited bleeding disorders, the sustained virologic response rate was significantly improved in patients treated

with interferon and ribavirin compared with those treated with interferon alone and adolescents treated with combination therapy had a significantly higher sustained response than adults on the same regimen [14].

The study of Franchini et al. showed that 33 HIV-negative haemophiliacs with interferon-nonresponsive chronic hepatitis C were treated with interferon alpha-2b (5 MU three times weekly) and ribavirin (1-1.2 g daily) for 12 months. At the end of therapy, normalization of alanine transferase occurred in 14/33 treated patients (42.4%) and HCV-RNA was cleared in 12 (36.4%). Eleven patients (33.3%) became sustained responders. Genotype 1 was the only factor associated with a poor response to therapy. This study showed that combination therapy is effective in haemophiliacs who did not respond to a previous interferon treatment [19].

Another study with 13 haemophiliacs with chronic hepatitis C infection detected that the combination therapy with IFN alpha and ribavirin yielded better results: four of eight patients still untreated with IFN alpha, one of two relapsers, and one of three non-responders to previous IFN alpha monotherapy achieved sustained virological response [18].

Twenty naive haemophiliacs patients were treated with interferon and ribavirin for 12 months. The results were normalization of aminotransferases with clearance of HCV-RNA, what occurred in seven (35%) patients. Therefore the combination of interferon and ribavirin is well tolerated by haemophiliacs, who achieve similar sustained response rates to non-haemophiliacs [21].

Despite being a small case series, our study results are consistent with previous research. Among predictors of a sustained virological response are: age below 40 years, non-genotype 1, lower HCV-RNA levels, the absence of cirrhosis and the adherence to the treatment [11, 17]. Our group of patients was younger, free of clinical signs of cirrhosis and with a good referred adherence to prescribed medication, what could explain the good response rates. Side effects did not limit medication and did not differ of those described by other groups of not-haemophiliacs. Hence, it follows that an estimated sustained point response of 50% (95% CI: 27–73%) is reassuring. A larger group of both naive and nonresponders HIV co-infected patients are under treatment with pegylated interferon in our haemophilia unit.

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