

Efficacy of Interferon A-2b Monotherapy in B-Thalassemics with Chronic Hepatitis C

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ABSTRACT

Background & Aims: Monotherapy with standard or pegylated interferon (PegIFN) remains the first-line treatment for HCV infection in patients with thalassemia major (β TM), although its long-term impact is still unknown. We aimed to assess the efficacy of IFN-a2b/PegIFN-a2b (one or multiple treatment sessions) and the predictors for sustained virological response (SVR) in HCV-infected β TM patients.

Methods: Between 11/1992 and 12/2013 [median follow-up: 165.5 months (8-237)], 48 β TM HCV-infected patients [19 males, median age: 22 years (12-45)], received IFN-a2b (n=34) or PegIFN-a2b (n=14). Twenty-three patients (47.9%) had a previous splenectomy; 13/40 (32.5%) patients had Ishak stage ≥ 4 and 21/40 (52.5%) had siderosis grade 3-4. HCV-genotype was available in 36 patients (genotype 1: 47.2%, 2: 5.6%, 3: 25%, and 4: 22%). IL28B genotype was determined in 37 patients by means of in-house real-time PCR (CC: 27%, CT: 62.2%, TT: 10.8%).

Results: Totally, 15/48 (31.3%) achieved SVR following the first treatment and 18/48 (37.5%) after multiple courses. Splenectomy (p=0.01) and fibrosis grade ≥ 4 (p<0.05) were negative predictors for SVR (first course), whereas splenectomy (p<0.05) and age >18 (p<0.02) for SVR after multiple courses. In HCV-genotype 1/4 (n=25), none of the patients with CT or TT IL28B genotype achieved SVR compared to 50% of the CC patients (p=0.004).

Conclusions: Interferon is an effective therapeutic option in HCV-infected β TM patients. IL28B genotype was a strong predictor for SVR, together with splenectomy, age and fibrosis.

Key words: thalassemia major – chronic hepatitis C – interferon – pegylated interferon – IL28B polymorphisms.

Abbreviations: β TM: thalassemia major; HCV: hepatitis C virus; IFN: interferon; PegIFN: pegylated interferon; SVR: sustained virological response; SNP: single-nucleotide polymorphism; EVR: early virological response; ETVR: end of treatment virological response; HAI: hepatitis activity index.

INTRODUCTION

Expectancy and quality of life have been substantially improved in patients with thalassemia major (β TM) as the result of the combination of appropriate red blood cell (RBC) transfusions and effective iron chelation treatment [1]. Consequently, new challenges have been faced with a longer lifetime span. Liver disease is an important cause of morbidity and mortality and hepatitis C virus (HCV) infection together with iron overload remain the main etiological

factors of chronic liver disease in this setting [2]. They represent independent risk factors for liver fibrosis progression and their concomitant presence results in increased morbidity and mortality, as the majority of HCV-negative patients with a low iron load do not develop liver fibrosis, whereas HCV positive patients more frequently develop advanced fibrosis [3] and liver cancer [4].

The medical treatment of HCV infection in non-thalassemic populations has advanced considerably in the last few years, with IFN-free regimens showing recently very promising results [5]. In the setting of thalassemic patients, monotherapy with standard interferon (IFN)/pegylated IFN (PegIFN) with or without ribavirin remains an option; however, recent consensus guidelines [5] suggest the use of sofosbuvir, simeprevir and/or daclatasvir considering that they do not aggravate anemia, although there are no published studies to evaluate the safety of these regimens in this population.

The nucleotide sequence near the *IL28B* gene on chromosome 19 is associated with increased frequency of sustained virological response (SVR) following treatment with PegIFN and ribavirin [6, 7] as well as of spontaneous resolution of HCV infection. Indeed, the single-nucleotide polymorphism (SNP) of the C or T allele at position rs12979860 is highly predictive for SVR in Caucasian patients with HCV-genotype 1 treated with PegIFN and ribavirin [8, 9], whereas recently, Di Marco et al. [10] showed that TT genotype of rs8099917 polymorphism and CC genotype of rs12979860 were independently associated with SVR in 114 HCV-infected thalassemic patients treated with IFNa.

The efficacy of antiviral treatment and the impact of treatment in this difficult to treat group of HCV patients remain unknown. Therefore, the aim of this study was to assess the efficacy of monotherapy with IFN-a2b or PegIFN-a2b (one or multiple treatment courses) in thalassemic patients with chronic hepatitis C and to assess predictive factors for achievement of SVR including *IL28B* genotype.

PATIENTS AND METHODS

Forty-eight multi-transfused β TM patients with chronic HCV infection who had received IFN-a2b or PegIFN-a2b as monotherapy since November 1992 up to December 2013 were evaluated (follow-up: 165.5 months; range 8-237). The participants were recruited from two Greek centres: a) the Thalassemia and Hemoglobinopathy Unit of the University Hospital of Patras (n=37) and b) the Department of Medicine and Research Laboratory of Internal Medicine of the Medical School of the University of Thessaly (n=11). All subjects consented to participate in the study at the time of the interview. The Ethical and Scientific Committees of the respective two centers approved the study protocol.

Eligible patients for evaluation in this retrospective study were those who satisfied the following criteria: a) diagnosis of β TM; b) seropositivity for antibodies to HCV (anti-HCV; AxSYM HCV version 3, Abbott, Germany) and elevated transaminase levels and/or histological findings of chronic hepatitis C and/or detectable HCV RNA in serum, and c) monotherapy with IFN-a2b (Intron A; Schering-Plough, Kenilworth, NJ, USA; n=34, 70.8%) or PegIFN-a2b (Pegintron; Schering-Plough, Kenilworth, NJ, USA; n=14, 29.2%). All patients were seronegative for human immunodeficiency virus (HIV) and immune against hepatitis B virus (HBV).

Chelation treatment

Blood transfusion requirements were evaluated before, during and following antiviral treatment in all treated patients. All patients were treated with deferoxamine until the year 2000. Afterwards, six of them continued with the combination deferoxamine + deferiprone since it was a more intensive regimen for those who were heavily iron-overloaded. Two of them exhibited severe neutropenia, and discontinued both IFNa and deferiprone [11]. Neutropenia occurred in another patient, which was resolved following a transient discontinuation of IFN treatment for two weeks. After that, the patient continued with IFN and completed the treatment schedule. After 2005, some patients were treated with oral deferasirox, and none

exhibited neutropenia or any other hematological toxicity. In addition, during the treatment period, no significant non-hematological toxicities were associated with the use of iron chelators, apart from one patient who received deferasirox and suffered from transient hearing loss. All patients had regular monitoring of serum ferritin (every two months). The patients with known liver iron content before the start of chelation treatment had repeated measurements of the liver iron content every year by Magnetic Resonance Imaging (MRI) according to recent practice guidelines [12].

Definitions of response

Early virological response (EVR), end of treatment virological response (ETVR), sustained virological response (SVR), relapse and non-response were defined according to the recent EASL clinical practice guidelines [5]. The histological response was defined as the difference of at least two points between the total Ishak necroinflammatory activity scores and of at least one point in the fibrosis score in paired biopsies performed before and at the end of treatment. Diagnosis of liver cirrhosis was based on histological findings or compatible clinical, laboratory, and imaging data.

Virological data

Serum HCV RNA was detected with a sensitive polymerase chain reaction assay (Cobas Amplicor, Roche Molecular Diagnostic Systems, Branchburg, NJ, USA). Serum HCV RNA was quantified by bDNA (Versant, Bayer Healthcare, Tarrytown, NY, USA). The HCV genotype was determined by Line Probe Assay (HCV Genotype Assay, Versant, Bayer Healthcare, Tarrytown, NY, USA).

Antiviral treatment

During the treatment period, the IFN dose was reduced or discontinued according to the Manufacturer Package Insert Recommendations. In cases of failure in achieving SVR, patients were considered to receive retreatment according to the current guidelines and patients' decision. Patients scheduled to receive treatment with IFN-a2b 3 MU/m² three times weekly or PegIFN-a2b (based on patient body weight) once weekly for a period of 48 weeks.

IL28B genotyping

IL28B genotype rs1297980 was determined by means of an in-house real-time PCR method in conjunction with minor groove binder probes. Each genomic DNA sample was amplified using TaqMan® Gene Expression Master Mix (Applied Biosystems, ABI) and specific primers and probes, corresponding to the respective SNP to be genotyped. The assay was carried out using the LightCycler® 480 (Roche), under the following conditions: 10 min at 95°C, 45 cycles: 10 sec at 95°C, 30 sec at 60°C, and 1 sec at 72°C. Automated allele calling was performed by means of endpoint genotyping software (Roche).

Histological data

Percutaneous liver biopsies were performed in 40 patients (83.3%). All specimens were examined by one pathologist (AT). In each biopsy, several histologic features were assessed, and the Hepatitis Activity Index (HAI) (modified HAI grading-

necroinflammatory scores) was applied as minimal (grade 1-2), mild (grade 3-6), moderate (7-12), or severe (13-18). In addition, architectural changes (modified staging-degree of fibrosis and presence/absence of cirrhosis) were recorded [13]. Iron accumulation was graded histologically on a scale 0-4+ according to a method described elsewhere [14].

Statistical analysis

Numerical data were expressed as median and range and categorical data as counts and percentages. All variables were tested for normal distribution using the Kolmogorov-Smirnov test. Categorical variables were tested using the chi-square and Fisher's exact test. Continuous variables with and without normal distribution were compared using Student's *t*-test or the Mann-Whitney U test, respectively. Paired *t*-test was used to assess possible associations between histological features of pre- and post-treatment liver biopsies. Two-sided *p* values less than 0.05 were considered as statistically significant. The SPSS statistical package (version 19.0 for Windows; SPSS Inc, Chicago, Illinois) was used.

RESULTS

The baseline characteristics of patients are shown in Table I.

Table I. Baseline characteristics of the study population

Age, years	22 (12-45)
Age >18 years	38 (79.2)
Male/Female	19/29
ALT (IU/L)	107 (33-331)
Duration of HCV infection (months)	16 (1-45)
Iron chelator use, n (%)	44 (91.7)
BMI (kg/m ²)	23.1 (17.3-30.4)
Serum ferritin (ng/ml)	1926.5 (373-10820)
Inflammation grade	6 (3-10)
Fibrosis grade	3 (1-6)
Fibrosis grade \geq 4, n (%)	13/40 (33.3)
Siderosis grade	3 (1-4)
Siderosis grade 3-4, n (%)	21/40 (52.5)
Steatosis, n (%)	10/40 (25.6)
Cirrhosis, n (%)	11 (22.9)
Splenectomy, n (%)	23 (47.9)
Genotypes 1/2/3/4, n=36, (%)	17/2/9/8 (47.2/5.6/25/22.2)
IL28B genotypes CC/CT/TT, n=37, (%)	10/23/4 (27/62.2/10.8)
Follow-up (months)	165.5 (8-237)

ALT: alanine aminotransferase, BMI: body mass index, IL28B: interleukin-28B. Data is given as median and range in parenthesis or as percentage; n=number of subjects tested.

Virological response

First treatment course

Thirty-four (70.8%) patients completed the treatment schedule. EVR and ETVR rates were 43.8% (n=21) and 37.5% (n=18), respectively. SVR was achieved in 15 of the 48 (31.3%) patients. Among 14 patients who did not complete

the treatment schedule, 3 (21.4%) achieved SVR. Reasons for treatment discontinuation were: increase in transfusion requirements (n=3), severe neutropenia (n=2), non-response (n=7), weight loss (n=1), and other (n=1). Twelve (85.7%) of these patients received treatment for 6 months or more. HCV genotype was available in 36 patients. Twenty-five of them (69.4%) were infected by HCV genotype 1/4; of these, 3 (12%) achieved SVR. In HCV genotypes 1/4, none of the patients with CT or TT *IL28B* genotype achieved SVR after the first treatment course compared to 33.3% of those with CC genotype (*p*=0.02) (Fig. 1). By contrast, no correlation was found between *IL28B* genotypes and treatment response rates in patients with HCV genotypes 2/3 (data not shown). The characteristics of patients who responded to the first treatment course are shown in Table II. Splenectomized patients (*p*=0.01) and patients with fibrosis grade \geq 4 (*p*<0.05) were less likely to achieve SVR. In the multivariate logistic regression analysis, only splenectomy was found predictive of not achieving SVR [odds ratio (OR) 9.76, 95% CI 1.03-92.59, *p*=0.047].

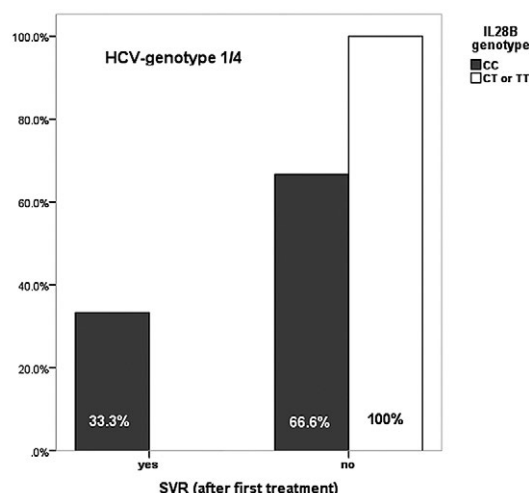


Fig. 1. Sustained virological response (SVR) rates after the first treatment course according to *IL28B* genotypes (CC or CT/TT) in patients with HCV genotypes 1 and 4 (*p*=0.02).

Retreatment

From those patients who failed to achieve SVR (n=33) with the first treatment session, 16 (48.5%, or 33.3% of the total number of patients) received multiple courses of treatment: 2 courses (n=10), 3 (n=4), 4 (n=1), and 5 (n=1). Retreatment resulted in SVR in 3 of them (18.8%). None of the cirrhotic patients (n=3) who received IFN as initial treatment achieved SVR with retreatment using PegIFN. Non-responders compared to relapsers (*p*<0.02) and patients with *IL28B* genotype other than CC (*p*=0.01) were less likely to achieve virological response following retreatment (Table III). In the multivariate analysis, none of the factors were found predictive for SVR following retreatment.

End of follow-up

At the end of follow-up, 18 patients in total (37.5%) achieved SVR after one or multiple treatment courses

Table II. Characteristics of patients with or without sustained virological response (SVR) after the first treatment course

	SVR (N=15)	No SVR (N=33)	P value
Age (years)	20 (15-38)	23 (12-45)	0.185
Age>18 (n)	10	28	0.151
Male sex (n)	5	14	0.551
Iron chelator use (n)	14	30	0.778
Cirrhosis (n)	3	8	0.746
Splenectomy (n)	3	20	0.01
Genotype (1/4 vs 2/3) (n)	3/3	22/8	0.257
IL28B genotype CC (n)	4	6	0.406
IFN/PegIFN (n)	12/3	22/11	0.346
Treatment discontinuation (n)	3	11	0.346
Inflammation grade	5 (3-9)	6 (3-10)	0.4
Fibrosis grade \geq 4 (n)	1	12	<0.05
Siderosis grade 3-4 (n)	8	15	0.486
BMI (kg/m ²)	23.875 (17.3 – 30.4)	22.735 (17.4 – 28.4)	0.405
Ferritin (ng/ml)	2144 (374 – 7868)	1887 (669 – 10820)	0.874
PLT (x10 ⁹ /L)	277.5 (152 – 588)	284 (109 – 884)	0.792
Bilirubin (mg/dl)	1.4 (1.2-1.9)	1.42 (0.6 – 7.5)	0.735
INR	1.26 (1.15-1.48)	1.13 (1-1.46)	0.079
ALT (U/L)	83.5 (33-194)	111 (40-331)	0.176
γ -GT (U/L)	23 (13-114)	41 (14-93)	0.133
ALP (U/L)	106 (67-228)	87 (46-514)	0.568

ALT, alanine aminotransferase; IFN, interferon; PegIFN, pegylated interferon; BMI, body mass index; IL28B, interleukin-28B; PLT, platelets count; INR, international normalized ratio; γ -GT, gamma-glutamyl-transferase; ALP, alkaline phosphatase. Data is given as median and range in parenthesis; n=number of subjects tested

including 5/25 (20%) patients with HCV genotype 1/4. Of note, none of the patients with HCV genotype 1/4 and the CT or TT *IL28B* genotype achieved SVR compared to 50% of patients with the CC genotype ($p=0.004$) (Fig 2). On the contrary, no correlation was found between *IL28B* genotypes

and treatment response rates at the end of follow-up in patients with HCV genotypes 2/3 (data not shown). Patients with previous splenectomy ($p<0.05$) and those older than 18 years ($p<0.02$) at the initiation of treatment were less likely to achieve SVR at the end of follow-up (Table IV). In the multivariate analysis, neither age nor splenectomy was associated with SVR at the end of follow-up.

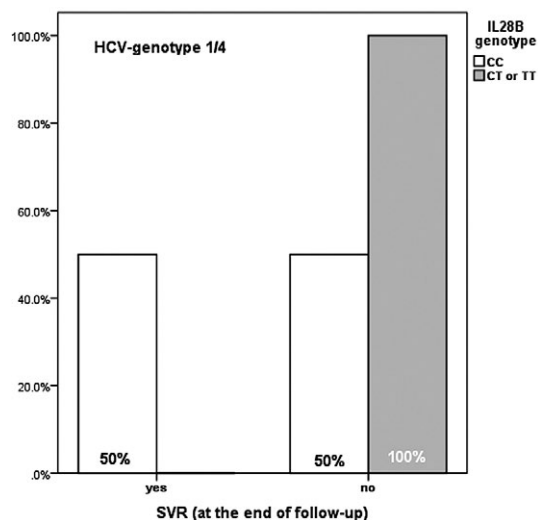


Fig. 2. Sustained virological response (SVR) rates at the end of follow-up according to *IL28B* genotypes (CC or CT/TT) in patients with HCV genotypes 1 and 4 ($p=0.004$)

Histological response at the end of treatment

According to the pre-treatment HAI, 27 patients had mild histological activity, whereas 13 moderate chronic hepatitis. No patient had severe inflammatory activity. Mild fibrosis (stage 1-3) was present in 26 of 40 (65%) patients, moderate (stage 4) in 3 patients (7.5%), incomplete cirrhosis (stage 5) in 8 (20%), whereas cirrhosis (stage 6) in 2 (5%) patients. Siderosis grade 3+/4+ was present in 21 (52.5%) patients. Post-treatment paired liver biopsies were available for 15 of 40 (37.5%) patients. There was a significant decrease in the fibrosis grade after the completion of treatment ($p<0.05$), as well as lower inflammation and siderosis grade but without statistical significance ($p=0.093$ and 0.065 , respectively). However, in the cohort of patients with SVR at the end of follow-up, there was a significant improvement in inflammation ($p<0.03$) and fibrosis grade ($p=0.03$), but again siderosis grade remained unaffected after treatment ($p=0.148$). Histological response at the end of follow-up was found in 33.3% of the patients (5 of 15 patients with paired liver biopsies).

Table III. Characteristics of patients with and without sustained virological response (SVR) after retreatment

	SVR (N=3)	No SVR (N=13)	P value
Age (years)	17 (15-30)	24 (13-43)	0.312
Age>18 (n)	1	11	0.064
Male sex (n)	1	6	0.687
Iron chelator use (n)	3	12	0.62
Cirrhosis (n)	0	3	0.356
Splenectomy (n)	2	9	0.77
Genotypes (1/4 vs 2/3) (n)	2/0	8/4	0.334
IL28B genotype CC (n)	1	0	0.01
IFN/PegIFN (n)	2/1	11/2	0.473
Non-responders/relapsers (n)	1/2	12/1	<0.02
Inflammation grade	5 (4-6)	6.5 (3-10)	0.51
Fibrosis grade ≥ 4 (n)	1	5	0.99
Siderosis grade 3-4 (n)	0	3	0.425
BMI (kg/m ²)	21.484 (21.2-28.4)	23.567 (21.2-26.9)	0.545
Ferritin (ng/ml)	1657 (1235-2212)	1440 (887-2900)	0.245
PLT ($\times 10^9/L$)	266 (205-278)	405 (193-549)	0.513
Bilirubin (mg/dl)	1.1 (0.9-1.2)	1.6 (1.1-1.7)	0.127
INR	1 (1-1.2)	1.13 (1.05-1.2)	0.6
ALT (U/L)	125 (93-201)	100 (83-194)	0.127
γ -GT (U/L)	32 (13-56)	44 (18-93)	0.127
ALP (U/L)	105 (97-277)	82 (49-180)	0.127

ALT, alanine aminotransferase; IFN, interferon; PegIFN, pegylated interferon; BMI, body mass index; IL28B, interleukin-28B; PLT, platelets count; INR, international normalized ratio; γ -GT, gamma-glutamyl-transferase; ALP, alkaline phosphatase; Data is given as median and range in parenthesis; n=number of subjects tested.

Development of cirrhosis

Eight (16.7%) patients developed cirrhosis during follow-up although two of them achieved SVR after the first, and one after two treatment courses. One patient from this cohort developed cholangiocarcinoma after 152 months.

DISCUSSION

The main finding of this cohort, observational study was the achievement of SVR in 37.5% of HCV-infected patients with β TM treated with one or multiple courses of IFN/PegIFN monotherapy. Predictive factors for treatment failure were older age, splenectomy and advanced fibrosis in liver histology, as reported in previous studies [15-17]. However, we also evaluated the impact of *IL28B* genotypes on treatment response, and we found that as in non-thalassemic HCV patients, CC genotype was positively associated with SVR in our β TM HCV-infected patients. So far, there are only a few reports on the potential impact of *IL28B* genotypes in β TM HCV-infected patients [10, 18]. Furthermore, another strong advantage of this study is the long follow-up period (median 165.5; range 8-237 months) compared to other trials [15, 19-23]. Indeed, to the best of our knowledge, this is the study with the longest follow-up aiming to evaluate the impact of antiviral treatment for HCV infection in this setting.

The reported virological response rates among β TM patients with HCV are similar [20, 24] or even better than

in non-multi-transfused patients. Actually, many previous studies favour the treatment of chronic HCV infection in this population [19-22], particularly in those with non-advanced liver disease, reporting SVR rates up to 53% (65.1% in genotype 1b but 16% in cirrhotics) [21], or even higher in children [22]. This could be explained by the lower median age of thalassemic patients and the lower incidence of cirrhosis. However, in our present report the SVR rate (37.5%) was lower than that recorded in some previous monotherapy studies (SVR rates range: 40-80%) [9, 16, 19, 21, 22, 25-28]. This discrepancy could be attributed to the high percentage of cirrhotics (22.9%), the high proportion of HCV genotypes 1 and 4 (69.4%) and the relatively high rate of treatment discontinuation (29%) in our study cohort, although half of the patients who discontinued prematurely were non-responders to antiviral treatment.

In our study, all patients received monotherapy as the first-line treatment because in thalassemic patients the haemolytic effect of ribavirin is of major importance [15, 22]. Small studies on selected patients with combined treatment of IFN/PegIFN and ribavirin have reported good SVR rates (range 33-72%) accompanied, however, by a 30-50% increase of transfusion requirements [12, 18, 23, 29-31]. In addition, a recent meta-analysis in β TM patients [31] showed that the pooled odds ratios of SVR for genotype 1 versus non-genotype 1 patients were 0.46 (95% CI: 0.22-0.95) in IFN monotherapy and 1.7 (95% CI: 0.46-6.04) in ribavirin/IFN combination therapy. In contrast to the latter findings, a recent randomized controlled

Table IV. Characteristics of patients with and without sustained virological response (SVR) at the end of follow-up

	SVR (N=18)	No SVR (N=30)	P value
Age (years)	20 (15-38)	23 (12-45)	0.08
Age>18 (n)	11	27	<0.02
Male sex (n)	6	13	0.493
Iron chelator use (n)	17	27	0.59
Cirrhosis (n)	3	8	0.425
Splenectomy (n)	5	18	<0.05
Genotype (1/4 vs 2/3) (n)	5	20	0.629
IL28B genotypes CC (n)	5	5	0.249
IFN/PegIFN (n)	14	20	0.412
Inflammation grade	5 (3-9)	6 (3-10)	0.293
Fibrosis grade \geq 4 (n)	2	11	0.093
Siderosis grade 3-4 (n)	8	15	0.988
BMI (kg/m ²)	23.55 (17.3-30.4)	22.8 (17.4-27.7)	0.519
Ferritin (ng/ml)	2178 (373-7868)	1783.5 (669-10820)	0.965
PLT (x10 ⁹ /L)	266 (152-588)	302 (109-884)	0.78
Bilirubin (mg/dl)	1.4 (0.9-1.9)	1.44 (0.6-7.5)	0.869
INR	1.26 (1.15-1.48)	1.13 (1-1.46)	0.079
ALT (U/L)	107 (33-201)	107 (40-331)	0.457
γ -GT (U/L)	23 (13-114)	41.5 (15-93)	0.4
ALP (U/L)	120 (67-277)	84.5 (46-514)	0.274

ALT, alanine aminotransferase; IFN, interferon; PegIFN, pegylated interferon; BMI, body mass index; IL28B, interleukin-28B; PLT, platelets count; INR, international normalized ratio; γ -GT, gamma-glutamyl-transferase; ALP, alkaline phosphatase; Data is given as median and range in parenthesis; n=number of subjects tested.

trial (RCT) reported higher SVR rates in the group of patients who received monotherapy with IFN- α 2b compared to those treated with IFN/ribavirin combination (90.9% vs 44.4%, $p=0.049$) [32]. Nevertheless, according to the results of the aforementioned meta-analysis [31], a definite conclusion regarding the most appropriate therapeutical approach cannot be drawn considering the usually small number of β TM patients included in the relevant studies and the lack of randomised, comparative studies of IFN monotherapy, PegIFN monotherapy and their combination with ribavirin.

Retreatment was successful in 19% of our patients. Available data on retreatment of non-responder thalassaemics is scarce with SVR rates ranging from 0-100% suggesting that large RCTs are needed in an attempt to define the appropriate therapeutic schedule in this difficult-to-treat cohort [20, 23, 33, 34].

The histological response rate in our study was 33%. Actually, a significant improvement in the fibrosis stage in patients with paired biopsies before and after treatment completion independently of virological response was observed. In contrast, the inflammation and hemochromatosis grade although improved, did not reach statistical significance. However, in the cohort of patients with SVR, both inflammation and fibrosis scores were significantly improved. In the study of Di Marco et al. [19], periportal and portal mononuclear infiltration and piecemeal necrosis were reduced in all patients. Lobular activity did also decrease in some patients, while the degree of siderosis was essentially unaffected. In another trial

[35], an overall reduction of inflammation and necrosis is reported in responders to IFN monotherapy after bone marrow transplantation for homozygous β TM, whereas Donohue et al. [20] reported a significant improvement in periportal necrosis and portal inflammation, but intralobular degeneration and fibrosis or cirrhosis scores did not change. Consistent with our results, two Greek studies [16, 22] have shown a decrease in hepatic inflammation and fibrosis in complete responders though in one of them [16] treatment had no effect on staging.

So far, many host and viral factors have been identified as predictors of response in non-multi-transfused HCV-infected patients [5, 7]. In contrast, data on predictors of SVR in β TM patients is scarce [10, 15-17, 20-22, 29, 33, 36, 37]. In our study, splenectomy, advanced fibrosis and age older than 18 years were negative predictive factors for SVR similarly to other reports [16, 19]. Since splenectomy is usually performed in patients with increased transfusion requirements, it is possible that the statistical association of splenectomy with adverse treatment outcome reflects advanced disease with iron overload. Young age is associated with mild histological findings and absence of advanced fibrosis or cirrhosis, which predict positive response to antiviral treatment. Moreover, the CC *IL28B* genotype in HCV genotypes 1/4 but not in HCV genotypes 2/3 thalassaemic patients was a significant predictor of treatment response, both in naïve and treatment-experienced patients, similarly to non-multi-transfused patients [7-9].

Limitations of our study are the relatively small sample size with only 15 patients having paired liver biopsies, and the

retrospective design. However, all patients were followed for a long time with no one lost to follow-up, and the results were consistent to most of the larger studies [10, 15, 17, 19, 21, 23, 29, 38, 39], whereas very few studies [16, 19, 20, 22] report data from paired biopsies in this setting.

A large number of novel antiviral agents, including direct-acting antivirals and host-targeted agents, have been introduced in the treatment of HCV with pangenotypic SVR rates far exceeding 90% [5]. New treatment strategies are currently under development including IFN-containing or IFN-free regimens. The new treatment options are easier to administer, have fewer adverse events and contraindications, and shorter duration. The new EASL guidelines suggest that IFN-free regimens such as sofosbuvir, simeprevir and/or daclatasvir should be used in patients with hemoglobinopathies, considering that, in contrast to IFN and ribavirin, they do not aggravate anaemia [5]. However, there are no clinical trials of the new agents published in this population. This, together with the high cost of the new oral antiviral agents [40], demands further research in this setting of patients. Moreover, according to our previous experience [41], survival of patients with β TM is mainly associated with heart failure, non-adherence to chelation treatment and non-HCC malignancies rather than with HCV infection or anti-HCV treatment. Thus, comorbidity, treatment compliance and potential adverse events should be considered before taking the decision to initiate anti-HCV treatment.

CONCLUSIONS

Interferon monotherapy is an efficient therapeutic option in HCV-infected β TM patients. Splenectomy, older age, advanced fibrosis and certain *IL28B* genotypes are associated with poor response to antiviral treatment. However, current literature lacks sufficient evidence to safely conclude on the best therapeutic regimen. Thus, larger studies are warranted to evaluate better the need, efficacy and safety of antiviral therapy in this cohort of patients, as well as firstly, to define the criteria of anti-HCV treatment initiation and secondly, to determine the subpopulations of thalassemic patients that are more likely to benefit from treatment.

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