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Liver fibrosis regression in patients with β-thalassemia major following hepatitis C treatment with direct-acting antivirals (DAAs)

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Abstract

Background: Infection with hepatitis C virus (HCV) and transfusion-induced iron overload are the main causes of liver disease in patients with β-thalassemia major (β-TM). Direct-acting antivirals (DAAs) have improved the management of chronic hepatitis C achieving high rates of sustained virological response (SVR). However, there are limited data concerning the influence of DAAs on fibrosis progression in this setting. The aim of this study was to examine the impact of DAAs treatment on liver fibrosis in patients with chronic hepatitis C (CHC) and β-TM with the utilization of transient elastography (TE).

Methods: Between 1/2015 and 7/2019 [median follow-up: 35 months (IQR range: 24-36.5)] 11 β-TM HCV-infected patients [median age: 46 years (IQR range: 40-57); genotype 1/2/3/4: 9.1/9.1/45.4/36.4%] who received DAA-based treatment were evaluated. All patients were under regular iron chelation treatment. The stage of liver fibrosis was determined using transient elastography (TE).

Results: Overall SVR rate after treatment with DAAs was 100% (11/11). Median liver stiffness at first year of follow-up (range: 6-12 months) was significantly decreased compared to baseline value (6.7 kPa vs 10.3 kPa; p= 0.013). Improvement of liver stiffness measurements (LSMs) in 4 patients corresponded to reversal of cirrhosis according to predefined TE cut-off values. All but two patients attained decreased TE values in their post-SVR examinations. No significant change was observed in 5 patients who were re-assessed at 12-48 months post-treatment (5.6 kPa vs 6.7 kPa; p=0.461).

Conclusion: DAAs treatment is a highly effective therapeutic option in HCV-infected β-TM patients regarding its effect on liver fibrosis.

Key words: *Thalassemia; chronic hepatitis C; liver cirrhosis; elastography; direct-antiviral agents*

INTRODUCTION

β-Thalassemia major or Cooley's disease (β-TM) is an inherited genetic disorder, caused by impaired synthesis of beta chains of the hemoglobin tetramer [1]. A serious consequence of the disease is iron overload, induced by regular transfusions, necessary for the maintenance of normal growth and development, and by the paradoxical suppression of hepcidin production [2,3]. One of the

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main target-organs affected by iron toxicity is the liver and hepatic fibrosis has been reported to correlate with transfusion burden and liver iron concentration (LIC) [4].

Hepatitis C virus (HCV) is a prominent transfusion-transmitted infection, affecting approximately 71 million people globally, that causes chronic inflammation with complications, such as liver cirrhosis and hepatocellular carcinoma (HCC) [5]. Notably, the prevalence rate of HCV infection in β-TM patients is recorded to be as high as 40% in our region, varying widely among international studies [6-8]. Apparently, individuals with β-TM and HCV infection represent a special group with two concomitant independent risk factors to develop advanced liver disease [9].

Preceding the introduction of direct-acting antivirals (DAAs), interferon (IFN) based regimens and ribavirin were the standard of care in HCV infection. Patients with β-TM were a population difficult to cure, with poor response to treatment and higher HCV reactivation rates [10-13]. In addition, ribavirin administration induces increased transfusion requirements and elevated body iron accumulation [14,15]. DAAs revolutionized the treatment against HCV, achieving unprecedented rates of sustained virological response (SVR>90%) without significant adverse effects [16,17].

However, the progression of liver fibrosis following treatment with novel agents in patients with β-TM has not yet been thoroughly clarified. Transient elastography (TE) is a non-invasive diagnostic tool, used to assess liver stiffness, and a surrogate marker of fibrosis, through analysis of vibration generated mechanical waves. Markedly, TE measurements are not influenced by liver iron deposition and provide satisfactory accuracy in predicting hepatic fibrosis in HCV-infected patients with β-TM, comparable to liver biopsy [18-21].

The aim of this study was to assess the impact of DAAs on liver fibrosis in β-TM patients with chronic HCV infection with the utilization of TE.

MATERIALS AND METHODS

Study population

In this observational study registering real-world experience, recruitment took place in the University Hospital of Patras in a period from January 2015 up until July 2019. Inclusion criteria were: 1) transfusion-depended, well-established β-TM 2) chronic hepatitis C (CHC) infection, defined by positive HCV RNA test for at least 6 months, and 3) treatment with DAAs. Patients with hepatitis B virus (HBV) or human immunodeficiency virus (HIV) co-infection were excluded. All patients were under regular chelation therapy, according to their individual features. Once the specific population of the study was defined (n=11), analysis of baseline characteristics was performed. Response to treatment was assessed and, subsequently, liver stiffness progression was evaluated using TE.

Ethical considerations

All study participants, or their legal guardian, provided informed written consent prior to study enrollment. The study protocol was reviewed and approved by the Ethics committee of the University Hospital of Patras. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki for medical research involving human subjects.

Patient assessment

Comprehensive medical history, complete blood cell count and blood biochemistry analysis were performed in all patients at baseline. Physical examination and monitoring of adverse effects were carried out at each visit.

HCV RNA was quantified by a highly sensitive viral load assay, the Versant HCVRNA 1.0 Assay (kPCR) - Siemens Healthineers, a real-time kinetic polymerase chain reaction (kPCR). HCV genotype was identified by Versant HCV Genotype 2.0 Assay, Line Probe Assay (LiPA) - Siemens Healthineers, a reverse-hybridization technology designed to identify HCV genotypes 1-6 and HCV subtypes. Quantitative detection of HCV RNA and HCV genotype identification tests were performed in the Microbiology Laboratory of the University Hospital of Patras, as a standard process in the Thalassemia Center patients. SVR was defined as undetectable HCV RNA, with a limit of detection <13 IU/ml in serum sample, 12 weeks after the therapy's cessation and it was used for determining patients' response to treatment [22,23]. Diagnosis of cirrhosis was based on solid clinical, biochemical, radiological or histological findings. T2* Magnetic Resonance Imaging was performed for the estimation of the LIC, applying the formula 0.202+25.4/T2* adapted from Wood et al. [24] Liver fibrosis status was evaluated before the administration of DAAs using TE (Fibroscan®; Echosens, Paris, France). Measurements were performed in the Gastroenterology Department of the hospital. The right hepatic lobe was targeted through an intercostal space access while the patient remained in the dorsal decubitus position with the right arm in maximal abduction. Using the ultrasound guide, the operator located a liver part of an adequate thickness of 6 cm or above without large vessels according to manufacturer's instructions. For all patients medium (M) size probe was used. The ratio between the number of valid

measurements and the total number measurements was used to describe the success rate. Only the examinations with at least 10 validated measurements, success rate of at least 60% and interquartile range (IQR) of less than 30% of the median TE value were considered reliable. The results were expressed in kilopascals (kPa). Cut-off values for diagnosing different stages of hepatic fibrosis were predefined. TE value ≥ 12 kPa was considered indicative of cirrhosis (F4), whereas a limit of 7.9 kPa was used to discriminate mild or no liver fibrosis (F0/F1) from moderate to severe fibrosis (F2/F3) [19,25]. All patients had their liver stiffness re-assessed within 6-12 months after the end of treatment.

Treatment

Treatment regimens were administered, taking into consideration the patient's HCV genotype, the availability of pharmaceutical agents and the Greek national and European guidelines for hepatitis C therapeutic intervention [22,26]. Patients were reimbursed for their treatment in accordance to the Greek national insurance system. Interferons had been the fundamental drug before the breakthrough of DAAs in the last decade and, thus, most of our patients (n=7, 63.6%) had received IFN-based treatment in the past.

In this study, five different regimens using DAAs were administrated: (i) SOF/LDV: A co-formulation of Sofosbuvir (SOF), a nucleotide analogue NS5B polymerase inhibitor, with Ledipasvir (LDV), a NS5A inhibitor; (ii) GRZ/EBR: A co-formulation of Grazoprevir (GRZ), a NS3/4 protease inhibitor, with Elbasvir (EBR), a NS5A inhibitor; (iii) OBV/PTV/r +DSV: A combination of Dasabuvir (DSV), a non-nucleotide analogue NS5B polymerase inhibitor, with a co-formulation of Ombitasvir (OBV), a NS5A inhibitor, with paritaprevir (PTV), a NS3/4 protease inhibitor, boosted by ritonavir (r); (iv) SOF/VEL: A co-formulation of Sofosbuvir (SOF) with Velpatasvir (VEL), a NS5A inhibitor; (v) SOF+DCV: A combination of Sofosbuvir (SOF) plus Daclatasvir (DCV), a NS5A inhibitor.

Deferoxamine was the first line agent of the chelation therapy in the majority of our patients. For patients exhibiting poor compliance, intolerance or side effects an alternative oral compound was used, either deferiprone or deferasirox. Combination of two chelators for undertreated individuals was in the physician's discretion.

Statistical analysis

Continuous variables were presented as medians (interquartile range, IQR). Frequency data were presented as absolute numbers and percentages, while comparison between categorical variables was performed with Pearson's chi-squared test or two-sided Fisher's exact test, when applicable. For non-parametric paired samples Wilcoxon rank sum test was applied. Statistical analysis was performed with the SPSS statistical software package (version 26.0; SPSS, Chicago, IL, USA). The threshold of statistical significance was set at 5% ($p \leq 0.05$).

RESULTS

Patients' characteristics

Fifty-four patients with transfusion depended β-TM and CHC infection, who received anti-HCV treatment, were identified in University Hospital of Patras. Among them, eleven individuals without HIV/HBV co-infection were treated with DAAs and, thus, were enrolled in our study. The main clinical and laboratory findings of these patients are presented in Table 1.

The most prevalent HCV genotype was genotype 3 (n=5, 45.4%), whereas genotype 1 and genotype 2 virus were each identified in one patient. Chelation therapy remained unchanged during the period of DAAs treatment and most of the patients received deferoxamine, as part of their chelation treatment (n=9, 81.8%). Although adherence was reported to be optimal, manifestations of iron deposition in endocrine glands, such as hypogonadism (n=6, 54.5%), diabetes (n=2, 18.2%) and thyroiditis/hypothyroidism (n=5, 45.4%), were observed in remarkable rates. Heart disease, a primary cause of mortality and morbidity in β-TM was recorded in 4 (36.4%) cases, while 4 other (36.4%) patients were diagnosed with liver cirrhosis.

Treatment

Seven patients with unsuccessful IFN-based therapy plus 4 patients, who had not received treatment in the past, were assigned to be treated with DAAs [median age: 46 (40-57), median follow-up: 35 months (IQR: 24-36.5)] (figure 1). SOF/LDV was administrated in 1 patient (9.1%), GRZ/EBR in 2 patients (18.2%), OBV/PRT/r+DSV in 1 patient (9.1%), SOF/VEL in 2 patients (18.2%) and SOF+DCV in 5 patients (45.4%). The selection of DAAs between individuals with different HCV genotypes is displayed in figure 2. All patients (n=11) responded to treatment and their serum HCV-RNA turned to be undetectable 12 weeks after the end of treatment (SVR=100%).

Baseline transient elastography

Prior to DAAs administration, liver fibrosis was assessed in all patients with TE and median liver stiffness was found 10.3 kPa (IQR 6.1-14.6). There were no cases of failure in the performance of TE, as 60% success rate and

Table 1. *Baseline characteristics of the patients.*

Age, years	46 (40-57)
Male sex, n (%)	4 (36.4)
Follow-up, months	35 (24-36.5)
BMI, kg/m2	23 (22-25)
HCV genotype, n (%)	
1	1 (9.1)
2	1 (9.1)
3	5 (45.4)
4	4 (36.4)
Chelation agent, n (%)	
Deferoxamine	4 (36.4)
Deferiprone	1 (9.1)
Deferasirox	1 (9.1)
Deferoxamine + Deferiprone	5 (45.4)
Cirrhosis, n (%)	4 (36.4)
Splenectomy, n (%)	8 (72.7)
MRI Liver Iron Concentration a, μg/g dry weight	4.2 (2.6-7.9)
IFN-experienced, n (%)	7 (63.6)
Fibroscan, kPa	10.3 (6.1-14.6)
AST, iu/ml	39 (35-76)
ALT, iu/ml	77 (33-138)
Bilirubin total, mg/dl	1.7 (1.4-2.5)
Albumin, g/l	45 (42-47)
Hemoglobin, g/l	10 (9.5-11.2)
Platelet count, x109/l	435 (258-506)
Diabetes, n (%)	2 (18.2)
Heart Disease, n (%)	4 (36.4)
Osteoporosis, n (%)	5 (45.4)
Hypogonadism, n (%)	6 (54.5)
Thyroidopathy, n (%)	5 (45.4)

Notes: Quantitative values are presented as median (interquartile range). a Data available on 6 patients.

Abbreviations: HCV, hepatitis C virus; BMI, body mass index; IFN, interferon; MRI, magnetic resonance imaging; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

IQR less than 30% of the median TE value were achieved in all patients. Applying the correspondence of TE values to stages of liver fibrosis, patients were classified in the following categories of liver damage: F0-F1/F2-F3/F4: n=4 (36.4%)/n=3(27.3%)/n=4(36.4%). Interestingly, patients previously treated with INF presented significantly higher baseline TE values in comparison with treatment-naïve patients [14.4 kPa (IQR 9.8-14.9) vs 5.75 kPa (IQR 4.45-9.3); p=0.038].

Furthermore, a T2* MRI was performed to quantify LIC in 6 patients and median value was found 4.2 mg/(g dry weight) (IQR: 2.6-7.9). In agreement with previous studies, no correlation was identified between LIC and TE results (r=0.123, p=0.816) [18-20].

Liver fibrosis regression

The effect of DAAs on liver fibrosis was evaluated using TE within the first post-treatment year (range: 6-12 months). Additionally, in 5 individuals liver stiffness was re-assessed during the following years of surveillance (range:12-48 months). Median TE value of the first post-

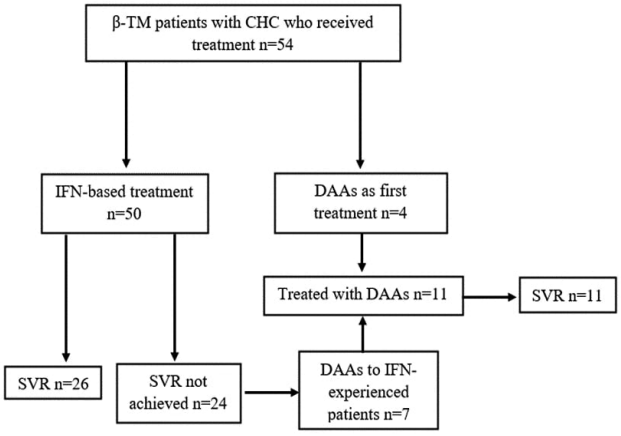

Figure 1. Flowchart of patients. β-TM: beta thalassemia; HCV: hepatitis C virus; CHC: chronic hepatitis C; IFN: Interferon; DAAs: direct-acting antivirals; SVR: sustained virological response.

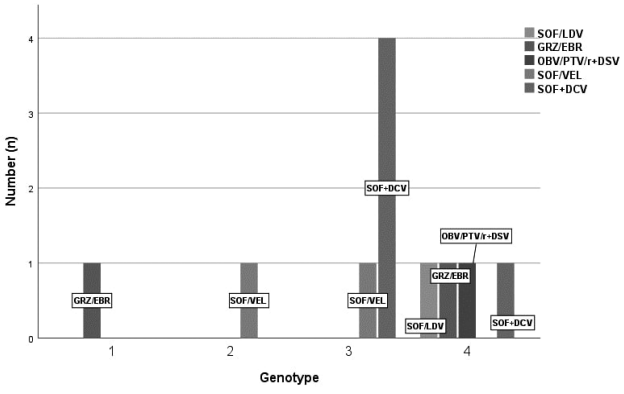

Figure 2. DAAs selection among patients with different HCV genotypes. DAAs: direct-acting antivirals; SOF: Sofosbuvir; LDV: Ledipasvir; VEL: Velpatasvir; DCV: Daclatasvir; GRZ: Grazoprevir; EBR: Elbasvir; OBV: Ombitasvir; PTV: Paritaprevir; r: Ritonavir; DSV: Dasabuvir.

treatment year was significantly decreased compared to baseline measurements [6.7 kPa (IQR: 4.8-8.8) vs 10.3 kPa (IQR 6.1-14.6); p= 0.013]. In patients with repeated post-treatment LSMs median TE remained unaltered during the following years [5.6 kPa (IQR: 5.2-8.3) vs 6.7 kPa (IQR: 4.8-8.8); p=0.461] (figure 3). Moreover, significant improvement of liver fibrosis was observed when the most recent LSMs of the patients [median liver stiffness was 6.7 kPa (IQR: 4.3-8.8)] were compared to their baseline values (p=0.016).

Notably, all but two patients had lower TE value in their most recent assessment, in comparison to their baseline measurement (figure 4). Even though 7 (63.6%) individuals had baseline liver stiffness corresponding to ≥F2 stage of fibrosis (≥7.9kPa), only 3 of them maintained a TE value above 7.9 kPa in post-treatment measurements (figure 5). Importantly all patients, who had pre-treatment LSMs indicative of cirrhosis (n=4), were classified as having an improved stage of liver fibrosis in their post-treatment TE examination (TE<12 kPa) (figure 4, 5).

Safety

All DAA-based treatment regimens were well-tolerated and no major adverse events were reported. Clinically significant drug-drug interactions between DAAs and chelation therapy were not observed. Adherence to treatment was optimal and early discontinuation was not recorded.

During the study follow-up, one 51 years-old male patient, who was diagnosed with hepatocellular carcinoma died.

DISCUSSION

The present study demonstrates that successful treatment with DAAs in β-TM patients with hepatitis C infection contributed to decreased post-treatment liver stiffness measurements (LSMs). DAAs appear to induce regression of hepatic fibrogenesis and even potential reversal of cirrhosis.

Various studies have provided estimates of liver fibrosis regression after DAAs treatment [27-38]. Our analysis focused on a particular subgroup of HCV infected patients, those with underlying β-TM. The key strength of our investigation is that the impact of DAAs was assessed with paired TE measurements in the course of a longer-term follow-up period compared to previous studies [27-38]. Post-treatment LSMs were carried out at least 6 months after the end of treatment (EOT). Besides, in certain cases, we conducted repeated post-treatment TE examinations over a period spanning

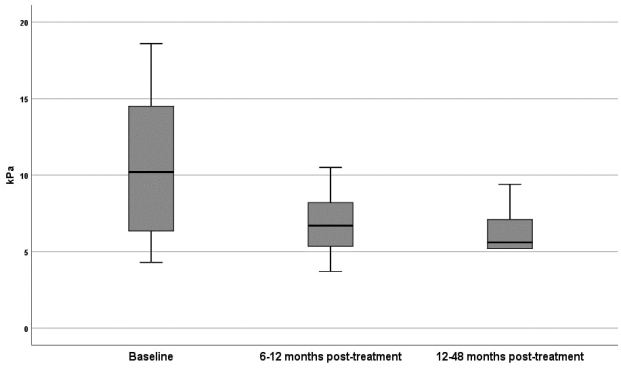

Figure 3. Boxplots of transient elastography measurements in patients: (i) before the administration of direct antiviral agents, (ii) during the first year after treatment, (iii) during the following years of monitoring.

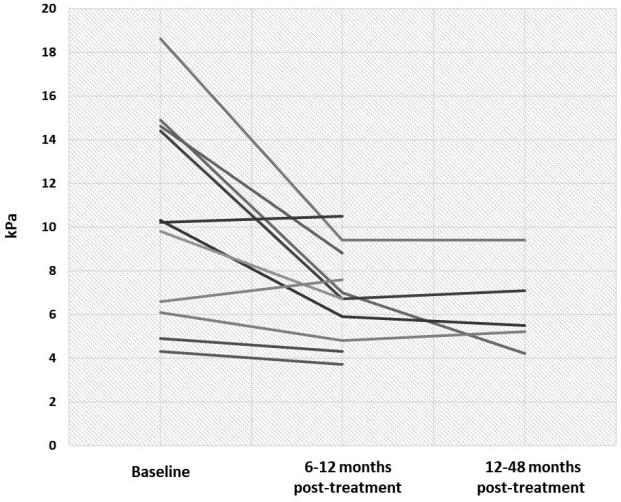

Figure 4. Liver stiffness measurements in patients (i) before the treatment with direct-acting antiviral agents, (ii) during the first year of follow-up, (iii) during the following years of monitoring.

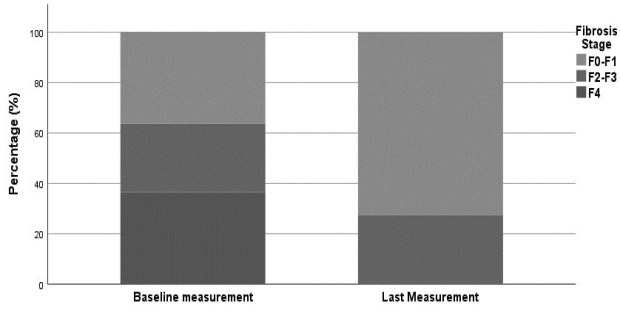

Figure 5. Classification of patients in groups corresponding to different stages of liver fibrosis in accordance with predefined transient elastography cut-off values. In the first column patients are categorized before the administration of direct-acting antivirals, while in the second column patients are categorized based on their most recent post-treatment liver stiffness measurement.

48 months. Hence, the continuing improvement of LSMs was appraised, allowing us to consolidate the assumption of liver fibrosis regression in patients treated with DAAs.

Liver stiffness change has been investigated in different categories of patients undergoing treatment with novel antivirals [27-38]. There is solid evidence that achieving SVR is related with decreased LSMs in accordance to our findings. More accentuated decline has been described in individuals with advanced liver disease [27,31-34,39]. In line with this, as portrayed in figure 4, our patients with baseline TE measurement equivalent to cirrhosis (>12 kPa) demonstrated improved stage of fibrosis in post-treatment LSM. Nevertheless, current data suggest that cirrhosis does not necessarily resolves in patients attaining SVR and might persist in a rate as high as 60%, potentially aggravated by the coexistent iron overload [38,40].

TE is a simple, noninvasive technique with satisfactory inter- and intra-observer reproducibility and has acquired an established role in the assessment of liver fibrosis in CHC by measuring hepatic stiffness [23,41]. D'Ambrosio et al. underlined that TE, although in post-eradication patients has lower sensitivity and, thus, lacks the ability to reliably exclude advanced liver disease, still remains a specific tool with high confirmatory strength in diagnosing cirrhosis. This might be attributable to the fact that, while cirrhosis is characterized by a shift from lobular to nodular architecture followed by annular fibrosis, in some cases nodular organization with trivial fibrous tissue is observed in post-SVR biopsies (bona fide cirrhosis) [40]. Obviously, TE retains its place as a reliable method for monitoring, versus consecutive invasive liver biopsies, which are plagued by sampling error and procedural risk of pain and hemorrhage. One basic factor for unreliable TE examination is obesity and particularly BMI >28 kg/m2. This explains the absence of invalid measurements in our study taking into consideration that our patients had a median BMI of 23 kg/m2 (IQR: 22-25) [42].

Whether lower LSMs mirror a regression of liver fibrosis remains controversial. It has been reported that the reduction of TE value correlates significantly with pre-treatment ALT level, a marker of necro-inflammatory hepatic activity [29,33,43]. In addition, a steeper decline of LSMs is witnessed during treatment rather than during the post-SVR period [29,38,39]. Hence, it has been supported that the observed improvement is mainly driven by suppression of liver inflammation due to viral eradication, rather than reversal of fibrotic histopathology. On the contrary, Chan et al. reported significant LSM decline between EOT and 12 months after, suggesting a combination of improvement in liver fibrosis and continued resolution of inflammation [31]. Moreover, studies comparing paired biopsies before and 6 months after IFN-based treatment indicate that a significant proportion (30-56%) of responders presented improved liver parenchyma histopathology [44,45]. Therefore, regression of fibrosis is possible as early as 6 months after achieving SVR. Our analysis was based on examinations that took place at least 6 months after the EOT and, possibly, depicts a degree of restored liver architecture without overlooking the parallel effect of the deescalated viremia-induced inflammation. However, patients with advanced liver disease in pre-treatment evaluation should be under close surveillance during follow-up because reduced TE values might be overestimated and not necessarily portray sufficient recovery of hepatic damage [46].

Transfusion-dependent β-TM cases with underlying HCV infection represent a challenging patient group for clinicians. Particularly, CHC and high liver iron concentration due to transfusions are two separate but co-existing risk factors for developing advanced fibrosis and, eventually, cirrhosis. The era of DAAs was accompanied with impressive rates of viral clearance even in populations with β-TM. Indeed, the highly effective treatment with DAAs in our patients (SVR=100%) was previously published as part of a large-scale Greek multicenter study [16]. According to the European Association for the Study of Liver (EASL) the IFN-free, ribavirin-free anti-HCV regimens for patients with hemoglobinopathies are recommended to be similar to standard treatment [23]. Besides, close monitoring is indicated after HCV eradication, due to residual danger of developing HCC [47]. There is a growing consensus that all patients with advanced fibrosis, namely F3 or F4 fibrosis before HCV treatment, should continue to be screened twice a year for HCC with a low-risk, noninvasive method such as ultrasound [47,48]. In a large retrospective study including 29,033 patients treated with DAAs, Ioannou et al. have reported that, except from cirrhotics, patients without cirrhosis but with FIB-4 scores ≥3.25 have a high enough risk to merit HCC surveillance, especially if FIB-4 remains ≥3.25 post-SVR [49]. This is crucial in β-TM patients, where underlying liver siderosis plays a pivotal role in fibrogenesis and consists a per se risk factor for end stage liver disease and HCC. In a recent panhellenic survey of neoplastic diseases, occurring among patients

with β-TM and other hemoglobinopathies, HCC was the most common cancer and was mainly attributed to the coexistent CHC infection [50]. Moreover, as reported by Triantos et al. poor adherence to chelation treatment, instead of antiviral therapy, is a predictor of worse prognosis in β-TM with CHC [10]. Therefore, implementation of regular monitoring and strict adherence to chelation treatment should be prioritized even after achieving SVR.

We acknowledge certain limitations of the current study. Firstly, the relatively small sample size reduces its statistical power and does not allow us to test specific parameters, such as cirrhosis, which might influence LSM improvement. However, β-TM constitute a small group between patients with CHC, implicating a demanding recruitment process. Furthermore, patients did not undergo sequential liver biopsies, which remain the benchmark of assessing liver fibrosis, in order to confirm histological alterations at time of LSMs. Nevertheless, the conduction of repeated post-treatment TE examinations during a long-term follow-up lessened the confounding effect of rapid regression of viremia-associated inflammation and confirmed the ongoing improvement of liver fibrosis.

In conclusion, the present study illustrates that treatment with DAAs in patients with β-TM is associated with significant improvement of TE values in line with current knowledge concerning other patient groups with CHC. We assume that this improvement, to a certain extent, accounts for a regression of hepatic parenchyma fibrosis without underestimating the parallel impact of the resolved inflammatory activity. Although reversal of cirrhosis can be hypothesized under circumstances, TE alone lacks the strength to confirm it. Patients with β-TM should be closely monitored after HCV eradication, especially those with advanced liver disease, since residual liver damage leading to the development of eventual hepatic complications is not yet fully elucidated. Longer follow-up period, larger cohort of patients and assessment of liver histology are some of the characteristics of additional studies that are essential in order to comprehensively interpret the long-term impact of novel antiviral therapy on patients with β-TM major.

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