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Impact of the direct-acting antiviral agents (DAAs) on chronic hepatitis C in Sardinian patients with transfusion-dependent Thalassemia major

Maria Laura Ponti[a], Federica Comitini[b], Debora Murgia[a], Roberto Ganga[a], Roberto Canu[a], Carlo Dessì[c], Maria Loreta Foschini[c], GianBattista Leoni[c], Maddalena Morittu[c], Maria Perra[c], Maria Paola Pilia[c], Maria Rosaria Casini[c], Antonietta Zappu[c], Raffaella Origa[c,*]

[a] *Medicine I, 'G.Brotzu' Hospital, Cagliari, Italy*
[b] *University of Cagliari, Italy*
[c] *Thalassemia Unit, 'A. Cao' Hospital, A.O. 'G. Brotzu', Cagliari, Italy*

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ABSTRACT

Background and aims: Direct antiviral agents (DAAs) have revolutionised the standard of care for the treatment of hepatitis even in patients with hemoglobinopathies. The aim of this study is to show how, thanks to DAAs, HCV infection has been substantially eradicated in one of the biggest Centres for the management of Thalassemia in Europe.
Methods: Thalassemia major patients regularly transfused and iron chelated in Cagliari (Italy) who were HCV-RNA positive were evaluated for the potential prescription of antiviral therapy.
Results: A total of 99 patients, 26 of whom had been diagnosed with cirrhosis, were treated with at least one dose of DAAs, which proved to be safe and well tolerated. Two of the patients died during the treatment after becoming HCV-RNA negative while another voluntarily interrupted the therapy. The final SVR in the patients who completed the treatment was 100%, while measuring 97% (96/99) in the Intention-to-Treat analysis. After DAAs, no new cases of hepatocellular carcinoma have been reported.
Conclusions: The use of DAAs in patients suffering from beta-Thalassemia major with chronic hepatitis C or cirrhosis can be considered safe and effective. Close monitoring for hepatocellular carcinoma development is, in any case, recommended indefinitely post-SVR.

1. Introduction

Beta Thalassemia major is an inherited, genetic blood disorder which affects the production of haemoglobin by the bone marrow.

Patients suffering from beta Thalassemia major require blood transfusions throughout their entire lives. Most of the Italian Thalassemia major subjects who received transfusion therapy before 1991 developed an active chronic hepatitis C infection [1,2].

In these patients, post-transfusional iron overload and HCV infection seem to be independent risk factors for liver fibrosis and their concomitant presence results in a greater risk [3].

Over recent decades, the progressive increase in life expectancy made possible by the significant decrease of heart-related mortality has led to liver disease becoming more prevalent [4]. Before the introduction of direct antiviral agents – DAAs –, patients with Thalassemia major were considered a difficult-to-treat population and, due to low response rates to IFN-based regimens, were perceived as being difficult-to-cure. The incidence of hepatocellular carcinoma (HCC) had been on the rise and diagnosis had typically been made at a significantly younger age when compared with the general population [5–7].

DAAs have revolutionised the standard of care for the treatment of hepatitis C [8–11]. Their use has been associated with high Sustained Virological Response (SVR) rates (>90%) and minimal side effects, even in patients with hemoglobinopathies [12–17].

The aim of this study is to show how, thanks to DAAs, HCV infection has been substantially eradicated in one of the biggest Centres for the management of Thalassemia major in Europe.

* Corresponding author at: Dott.ssa Raffaella Origa, Thalassemia Unit, 'A.Cao' Hospital, A.O 'G. Brotzu', Cagliari, Italy.
E-mail address: raffaella.origa@aob.it (R. Origa).

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2. Patients and methods

Data on patients with beta Thalassemia major and a documented history of HCV infection were collected and analysed using clinical case sheets and Webthal®, a web-based clinical records' Software program developed to help physicians in daily clinical patient management. All the patients registered on Webthal® gave their informed consent to the use of their clinical data for research studies.

All patients under study were regularly transfused and iron chelated at Day Hospital Talassemia Evolutiva—Ospedale Microcitemico 'A.Cao', Cagliari (Italy) according to the internationally accepted guidelines [18].

Those who proved to be HCV-RNA positive as of 1st January 2015 were evaluated in collaboration with the Hepatology Department — Medicina I, A.O. 'G. Brotzu', Cagliari (Italy) for the potential prescription of antiviral therapy based on available DAAs and according to the current guidelines for the treatment of HCV infection [9,10].

The following parameters were taken into account:

- HCV genotype;
- Previous treatment with interferon (INF) monotherapy or dual therapy Pegylated-IFN (PEG-IFN) and ribavirin (RBV);
- Stage of fibrosis determined by Transient Elastography (Fibroscan);
- Medical history, kidney and liver function lab tests and medications in use
- Concomitant cryoglobulinemia

Liver transient Elastography was performed using FibroScan (Echosens, Paris — France), considering an optimal cut-off of 7.7 kPa for mild fibrosis and of 13.0 kPa for cirrhosis, according to the meta-analysis made by Friedrich-Rust et al. [19]. In patients who started DAAs, blood samples were collected at baseline, after 1,2,3 and 4 weeks of treatment as well as at the end of the therapy. Adverse events were collected and a physical examination was carried out at each transfusion. SVR to therapy was defined as undetectable HCV-RNA by a quantitative real time PCR with a limit of detection of 15 IU/mL, and was assessed at week 12 after therapy (SVR12).

The data collected included levels of aspartate transaminase (AST), alanine transaminase (ALT), gamma glutamyl transferase, PT, serum creatinine, serum ferritin, quantitative serum HCV-RNA as well as the cryoglobulinemia course.

Ultrasound examination was performed at the start and at the end of the antiviral treatment, then quarterly for the first year after DAA therapy and yearly thereafter (biannually in cirrhotic patients).

The hepatic and cardiac iron overload was assessed prior to and post treatment with T2* magnetic resonance imaging (MRI, Type of scanner Siemens, software used CMRTtools, magnetic field 1.5 T). For the liver, the conversion from T2* to liver iron concentration (LIC) with the formula 0.202 + 25.4/T2* adapted from Wood et al. was used [20]. Liver iron overload ≥15 mg Fe/g d.w. was defined as severe, ≥7 and <15 as moderate, and ≥1.8 and <7 mg Fe/g d.w. as mild. Levels ≤1.8 mg Fe/g d.w. were considered normal.

Webthal® was used for the evaluation of the number of transfused units per month before, during and following the therapy.

Serum samples of patients who were non responders to treatment were sent to U.O.C. Virologia Molecolare, University of Rome — Tor Vergata for the resistance-associated mutations research on the aminoacidic region of NS3 protease (15-181aa), NS5A (1-185aa) and NS5B polymerase (92-576aa).

t-Test and ANOVA were performed to examine the changes in serum ferritin, ALT levels and other parameters between baseline, end of treatment and then 12 weeks later.

3. Results

3.1. Study population

Out of 208 patients with a documented history of HCV infection who were alive when DAAs became available in our Center, 101 were HCVRNA positive and 99 of them received at least one dose of DAAs from May 2015 to January 2018 (Fig. 1). Their baseline characteristics are described in Table 1. All these patients but four had

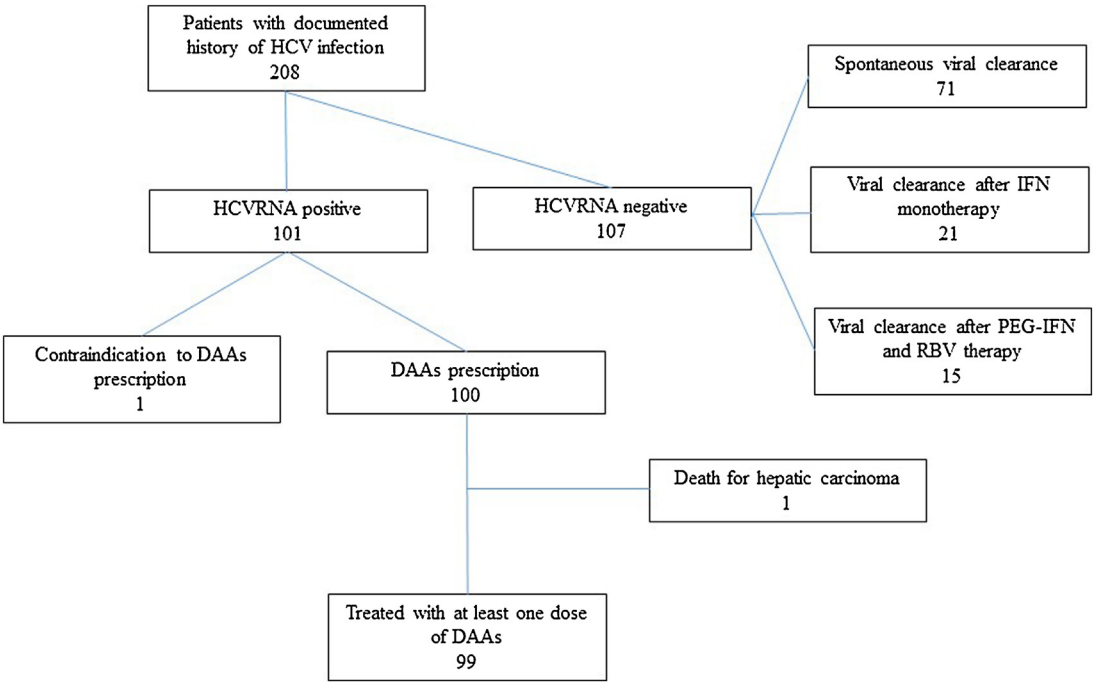

Fig. 1. Patients with chronic HCV infection alive as of January 2015. DAAs: direct antiviral agents; IFN: interferon; PEG-IFN: Pegylate-interferon; RBV: ribavirin.

Table 1
Baseline characteristics of thalassemia major patients treated with DAAs with or without ribavirin.

Median age, years (range)	40.2 (32.2–45.9)
Male sex, n (%)	54 (54.5%)
HCV genotype, n(%)	99
1a	5 (5%)
1b	56 (55.4%)
1a/1b	1 (1%)
1 unsubtypable	2 (2%)
2	24 (23.8%)
3	3 (3%)
4	8 (7.9%)
Cirrhosis, n (%)	26 (25.7%)
Treatment history	
Naïve, n (%)	63 (62.4%)
Interferon monotherapy	21 (21.2%)
Pegylated-Interferon + Ribavirin	15 (15.1%)
Median HCV RNA, range (log10 IU/mL)	565,785.5 (501–14,881,851)
Median ALT levels, range (U/L)	78.5 (14–322)
Median ferritin levels, range (μg/L)	1460 (282–8062)
Liver iron overload, n (%)[a]	
Absent	15 (12.6%)
Mild	49 (41.2%)
Moderate	17 (14.3%)
Severe	3 (2.5%)
Heart iron overload, n (%)[a]	
Absent	58 (48.7%)
Mild/moderate	24 (20.2%)
Severe	2 (1.7%)
Occult hepatitis B, n (%)[a]	40 (33.6%)
HIV infection, n (%)[a]	2 (2.4%)
Cryoglobulinemia, n (%)[a]	34 (28.6%)
Diabetes, n (%)[a]	14 (11.8%)
Heart disease, n (%)[a]	36 (30.2%)
Hypogonadism, n (%)[a]	49 (41.2%)
Hypothyroidism, n (%)[a]	24 (20.2%)
Hypoparathyroidism, n (%)[a]	11 (9.2%)
Osteoporosis, n (%)[a]	59 (49.6%)

[a] Data available in 84 patients.

been infected during their first years of life, before the introduction of the hepatitis C donor screening in 1991.

They were treated with various DAA regimens including in 6 cases RBV (Table 2).

Adherence to treatment was optimal in all the patients but one, who voluntarily withdrew after the first two doses.

3.2. Parameters of efficacy, virological response and follow up

ALT levels decreased from 99.6 ± 73.1 at baseline to 22.2 ± 14.6 IU/L at the end of treatment ($p < 0.0001$) and finally to 24.6 ± 17.4 IU/L at SVR evaluation ($p < 0.0001$).

End of treatment response was reached by 95 out of the 96 patients who completed one cycle of DAAs (98.9%) whereas 93 patients reached SVR. Of the remaining three, one was a cirrhotic patient who was treated with simeprevir plus sofosbuvir for 12 weeks and relapsed 12 weeks after the end of treatment. This patient was subsequently and successfully treated with a combination of sofosfuvir plus daclatasvir.

The other two patients, both non-responders, had been treated with ombitasvir/paritaprevir/ritonavir plus dasabuvir for 12 weeks.

The analysis of virus resistances showed the following mutations:

- Patient one: NS3 protease: D168DV; NS5A: Y93H; NS5B: S556R
- Patient two: NS3 protease: D168DV; NS5A: Y93H; NS5B: L159F; C316N: S55

Both of them were subsequently treated with the combination sofosbuvir/velpatasvir/voxilaprevir for 12-weeks finally reaching SVR.

Ultimately, the final SVR was 97% (96/99) in the Intention-to-Treat analysis and 100% (96/96) in the per-protocol analysis (Fig. 2).

To date, the patients have a mean follow up observation period of 668 days (range 87–1159 days) from starting on DAAs. None of the patients has developed hepatocellular carcinoma over this period of time.

The left ventricular ejection fraction (LVEF) was evaluated before and after DAA treatment in patients with cirrhosis: it remained unchanged (62.1 ± 7.8% vs 61.9 ± 8.4%, $p = 0.9$).

3.3. Efficacy and safety of DAAs in patients with HCV-associated cryoglobulinemia

Thirty-four patients had positive cryoglobulins at baseline, which were intermittently detected during the treatment. Eleven patients out of 16 whose regular determination of cryoglobulin was available became negative after beginning the antiviral therapy. Nine were negative at SVR (median time of negativization 12 weeks after the start of DAAs; range 4–24 weeks) while the other 2 proved negative after 28 and 72 weeks, respectively. Five patients still had positive cryoglobulinemia at the time of the analysis.

Clinical manifestations clearly ascribable to type I cryoglobulinemia were present in only one patient, a 44-year-old woman who had experienced repeated episodes of purpura of lower limbs with severe arthralgia and a painful peripheral neuropathy confined to the lower extremities with predominant sensory impairment for 10 years. Magnetic resonance imaging of the brain also revealed a cerebral vasculitis. After several cycles of NSAIDs and corticosteroids of very little benefit, she was treated in May 2015 with simeprevir and sofosbuvir for 12 weeks and, since she remained symptomatic, several cycles of plasmapheresis were performed and led to a significant improvement of the symptoms. Cryoglobulins finally became undetectable after 18 months from the start of antiviral treatment.

Table 2
Direct Antivirals regimens according to HCV genotype (99 patients).

1[a]	1a	1b	1a/b	2	3	4	Cycles (n)	Regimen
	1	7		15	3	1	27	Sofosbuvir + daclatasvir
1				3			4	Sofosbuvir + velpatasvir
		7				1	8	Sofosbuvir + ledispavir
				1			1	Sofosbuvir + ribavirin
		9				3	12	Sofosbuvir + simeprevir ± ribavirin
	1	2				1	4	Ledispavir ± ribavirin
1	1	4		4			10	Glecapevir + pibrentasvir
	1	26	1	1		2	31	Ombistavir/paritaprevir/ritonavir + dasabuvir ± ribavirin
	1	2					3	Grazoprevir + elbasvir
		2						Sofosbuvir + velpatasvir + voxilaprevir
2	5	59	1	24	3	8	102[b]	Total

[a] Genotype 1 non subtypable.
[b] Three patients received a second cycle of DAAs because they had not obtained SVR with the first one.

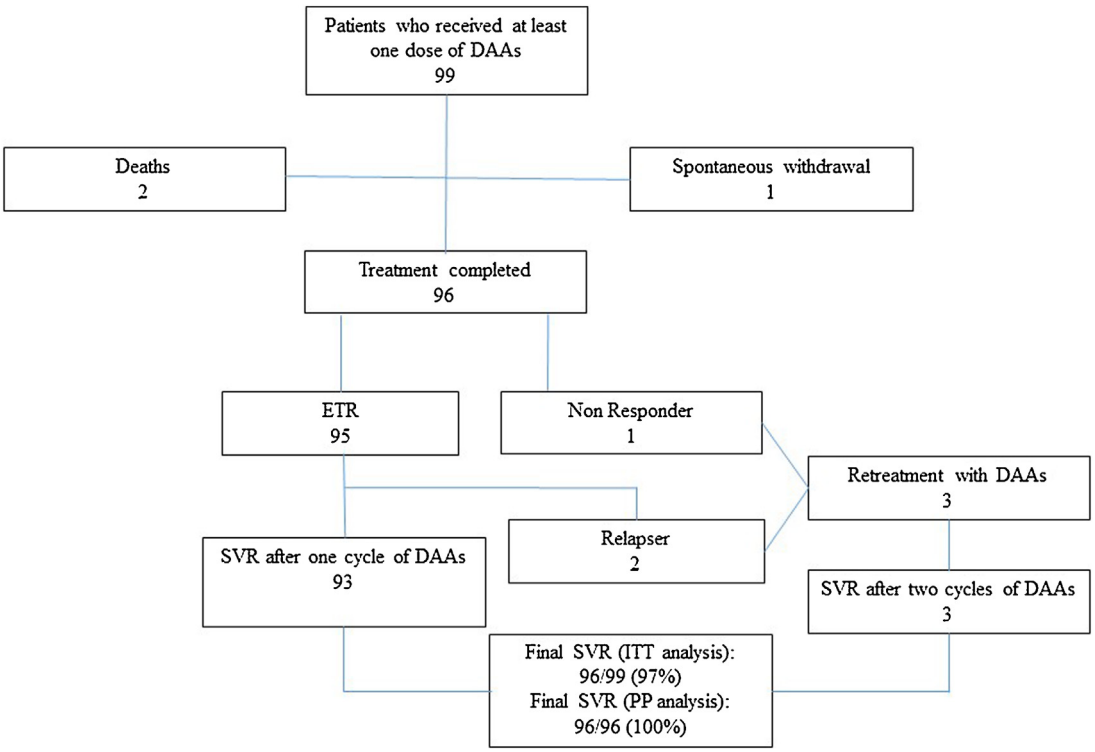

Fig. 2. Outcome of patients who received at least one dose of DAAs.
DAAs: direct antiviral agents; ETR: early termination response; ITT: Intention-To-Treat; PP: per protocol; SVR: Sustained Virological Response.

Table 3
Adverse events suspected of being related to the use of DAAs.

Adverse event	Grade[a]	n (%)[b]
No adverse events		56 (66,7)
Asthenia	1	13 (15,5)
Hypoalbuminaemia worsening	2	1 (1,2)
Anxiety	1	1 (1,2)
Nausea	2	1 (1,2)
	1	2 (2,4)
Headache	1	3 (3,6)
Stomach pain	1	2 (2,4)
Abdominal distension	1	1 (1,2)
Insomnia	1	1 (1,2)
Neutropenia	1	1 (1,2)
Vertigo	1	1 (1,2)

[a] NCI CTCAE version 4.0.
[b] Data referred to 84 patients.

3.4. Safety

Therapy with DAAs was discontinued prematurely in three patients; two of them, cirrhotic and showing severe iron overload and comorbidities, died after becoming HCV-RNA negative (one through multiorgan failure and the other through heart failure after a phase of hypoalbuminemia worsening). The third voluntarily interrupted the treatment after the first two doses because of a concomitant gastroenteritis, which based on clinical judgement was not associated with the antiviral therapy.

All treatment regimens were well tolerated and no serious adverse events were reported.

The most common findings were asthenia (13 patients; 13.1%), nausea (4 patients; 4%), and mild headache (3 patients; 3%) (Table 3).

Safety lab parameters did not significantly change during the treatment. Mean creatinine values were 0.7 ± 0.2 at baseline, 0.7 ± 0.2 at the end of the treatment ($p = 0.5$) and 0.8 ± 0.2 after 12 weeks ($p = 0.1$). Mean creatinine values remained stable also in patients chelated with deferasirox (0.8 ± 0.2 at baseline and 0.8 ± 0.2 at the end of the treatment; $p = 0.05$). All the patients concluded treatment without any dose-reduction caused by adverse events.

3.5. Chelation therapy, blood consumption, and iron overload parameters

During DAA treatment 77 (77.8%) patients were chelated with desferrioxamine. Most of them had changed their previous iron chelation therapy as a precaution due to the lack of data on drug interaction between DAAs and oral iron chelators.

Deferiprone was taken by only one patient and deferasirox by 21 (21.2%).

The need for blood transfusions did not change during the period of DAA therapy [4.6 units $\pm$ 1.2 before the treatment, 4.6 ± 1.1 at the end of the treatment ($p = 0.5$) and 4.7 ± 1.3 12 weeks after the end of therapy ($p = 0.4$)]. In patients treated with RBV, blood requirements increased from 4.7 ± 1.5 to 7 ± 2.6 ($p = 0.07$), but rapidly fell following the therapy (4.5 ± 1.2; $p = 0.5$).

At the same time serum ferritin levels dropped significantly during the treatment period (from 1872 ± 1573 to 1490 ± 1065 μg/L, $p = 0.0002$) while LIC remained unchanged (from 5.5 ± 4 to 5.2 ± 3.6 mg Fe/g d.w.).

4. Discussion

With the advent of non-invasive methods to measure organ iron before the appearance of clinical symptoms, new chelators and increased blood safety measures, the prognosis for individuals with beta Thalassemia has dramatically improved.

Since 2000, all of these developments have led to a significant trend in decreasing cardiac mortality which previously caused 71% of the deaths in individuals with beta Thalassemia major [21–23].

Recent studies in some European countries have shown that the number of patients who die from liver disorders now exceeds that of individuals who die from cardiac diseases [4].

In particular, the risk of hepatocellular carcinoma has progressively increased owing to liver viral infection, iron overload, and a longer life expectancy of the patients [5–7].

Treatment of chronic HCV in Thalassemia historically began with interferon (IFN) mono-therapy while the recommended treatment until a few years ago was a combination of PEG-IFN and RBV, which was not well-tolerated by thalassemic patients leading to both low compliance and SVR rates [24].

Moreover, RBV associated hemolysis determines an increase in the need for transfusions and the consequent worsening of iron overload leading in turn to an eventual progression of liver damage [25].

First-generation protease inhibitors, which were used in conjunction with PEG-IFN and RBV for the treatment of genotype 1 infection, were not experimented in Thalassemia patients because of their substantial adverse effects. In the end, the majority of patients infected with hepatitis C who did not clear the virus spontaneously were still HCV-RNA positive when DAAs became available in Italy. In accordance with the criteria previously laid down by the 'Agenzia Italiana del Farmaco (AIFA)' for the reimbursement of DAAs by the Italian National Health System, we initially treated patients with chronic HCV and severe extrahepatic manifestations of the disease and patients with ≥F3 fibrosis, and then the others. The fact that the treatment timeframe of all our patients spans from May 2015 means that we have been able to adopt almost all the therapies available over this period, and have obtained optimal results with all of them. As with other studies, we did not find significant differences in terms of SVR between patients with or without cirrhosis [12,15].

The majority of patients did not experience any side effects during the treatment, and the side effects reported were negligible in comparison with those depicted in previous antiviral therapies including IFN. The most relevant side effect was reported in one of the first patients treated when the combination of simeprevir, sofosbuvir and RBV for 12 weeks was associated with moderate nausea which required specific treatment with metoclopramide. The most commonly reported side effect was mild asthenia, a common finding in adults with Thalassemia for several reasons, and which in any case did not affect their daily-life during DAA therapy.

Finally, the effect of the new antiviral treatments on blood consumption was negligible even in the subgroup of patients treated with regimes containing RBV because of its reversibility and the brevity of the treatment.

Our results confirm therefore that the advent of DAAs has revolutionised the treatment of chronic hepatitis C even in subjects with Thalassemia major. Not only has the rate of premature withdrawal because of adverse events been reduced to virtually zero, while it was as high as 25% with IFN-containing regimens, but the probability of SVR has also dramatically increased from between 28 and 66% in patients under IFN monotherapy and between 31 and 93% in the largest cohort of patients treated with the combination PEG-IFN and RBV compared to results of close to 100% in patients treated with DAAs [25–27].

There has been much discussion about the role of iron overload in conditioning SVR in Thalassemia patients treated with PEG-IFN [28–30]. One international panel recommended that the intensification of chelation treatment be considered before starting antiviral treatment in patients with severe iron burden, although it underlined that there was little evidence of its utility in Thalassemia patients [24]. In our study the majority of patients had no liver iron or only a mild overload and, in any case, the results were equally positive in patients with moderate or severe liver iron overload so that the role of hepatic siderosis in conditioning SVR seems extremely unlikely.

Due to the lack of data about possible interactions between oral iron chelators and DAAs, we initially chose to put all the patients on desferrioxamine chelation for the period of treatment. After the publication of studies which demonstrated a lack of specific side effects and similar efficacy in patients treated with each of the iron chelators, we decided to continue with the original chelation therapy thereby verifying the data obtained by other Centers [12,15]. We were also able to confirm that renal function measured monthly in patients on deferasirox did not vary during DAA treatment either in terms of serum creatinine or in terms of tubular function.

There is an increased awareness of hepatitis B reactivation in chronic hepatitis C patients coinfected with HBV and treated with DAAs [31,32]. A recent systematic review and meta-analysis has shown that HBV reactivation and hepatitis caused by HBV reactivation may occur in patients with occult HBV infection and, although this does not affect SVR, HBV reactivation occurs earlier and is clinically more significant in patients with occult HBV who are treated with DAAs compared with IFN-based therapies [33]. Our patients were screened for overt or occult HBV infection before the beginning of DAA therapy. None of them tested positive for chronic hepatitis B while almost half of them had occult HBV. This is notably the first time that the prevalence of occult HBV has been reported in Thalassemia patients. When checking ALT on a monthly basis, we did not observe any ALT flare, and therefore HBV reactivation – despite the absence of formal evaluation – is extremely unlikely, in accordance with the literature on non-thalassemic patients.

LVEF was evaluated in patients with cirrhosis to assess whether or not the achievement of SVR can impact on cardiac function. Indeed, cirrhosis is considered a systemic disease [34] and a correlation between HCV infection and the risk of heart failure has been reported by some authors [25]. However, the majority of the cirrhotic patients under study had a normal LVEF at the start of the treatment and no correlations were apparent.

HCV is recognized as being both a hepatotrophic and lymphotropic virus and its replication in peripheral blood mononuclear cells may be etiologically implicated in HCV-related lymphoproliferative and immunological disorders. Among these conditions, B-cell clonal proliferative disorders such as mixed cryoglobulinemia (MC) and non-Hodgkin's lymphoma (NHL) have been shown to be strongly linked with HCV [35].

In 1995, a study aimed at determining the frequency of cryoglobulinemia and associated symptoms in 264 HCV-positive transfusion dependent Thalassemia patients registered the presence of cryoglobulines in 25.8% of cases [36], in line with our study which confirmed a high prevalence of cryoglobulinemia with only one patient presenting symptoms clearly linked to this condition. However, apart from the usual manifestations, cryoglobulinemia may be associated with symptoms such as asthenia, arthritis and proteinuria which are very common in Thalassemia patients and potentially associated with a number of causes, making their attribution to cryoglobulinemia difficult.

Although in our experience all patients with cryoglobulinemia treated with DAAs have reached SVR, not all of them exhibited negative cryoglobulines at SVR, as reported in non-thalassemic individuals [37,38].

Four patients at our Center notably developed non-Hodgkin-lymphoma before the introduction of DAAs and three of them died. All four were HCV-RNA positive and did not have MC (unpublished data).

In cryoglobulinemia vasculitis, B-cell proliferation may eventually reach an HCV-independent autonomous phase [38].

A long-term follow up on patients with Thalassemia major and MC treated with DAAs would therefore seem important, with

a periodic evaluation of lymphocytes subpopulation analysis, continuing even beyond the achievement of cryocrit negativity.

Liver stiffness measured with Transient Elastography was not used to evaluate changes in liver fibrosis after DAAs, since it is a well-known fact that it may be affected by a sudden decrease in liver inflammatory status [39].

In non-thalassemic subjects, the impact of DAA-based regimens on the occurrence of HCC in patients with cirrhosis, and in particular the recurrence of HCC following successful curative treatment is controversial.

Two large-scale prospective studies involving patients receiving DAAs in Italy have demonstrated that residual hepatocellular carcinoma risk is reduced and declines progressively over time after SVR [40,41]. In a meta-analysis including 26 studies (11,523 patients with cirrhosis) DAA therapy was not associated with a higher rate of occurrence of HCC [42].

Similarly, no new cases of HCC have been reported in our patients, although caution should be taken as our patient follow-up after DAA treatment was relatively brief. In the general population, post-SVR close monitoring for HCC development with liver imaging and alpha-fetoprotein is, in any case, recommended twice a year indefinitely at least in patients with HCV cirrhosis, since the long-term evaluation of the impact on the incidence of HCC is still ongoing [42]. This is even truer in patients with Thalassemia where other risk factors persist, the most relevant being obviously liver iron overload, which *per se* is a known risk factor for fibrosis and HCC.

Given the substantial improvements in the survival of patients with Thalassemia, it is still controversial whether the mild levels of iron overload that may be acceptable over a brief period could have long-term serious consequences [43].

According to some authors lowering iron levels even in patients who are not iron overloaded may reduce the risk of malignant transformation [44,45].

This reinforces the notion that an increased incidence of HCC in these patients cannot be excluded even after the eradication of HCV and, for this reason, it would seem prudent to maintain labile iron and total body iron levels within the normal range. This means that close ultrasound-monitoring remains mandatory in this population, especially but not only in patients with cirrhosis or significant iron overload.

In conclusion, our work shows how hepatitis C has been substantially eradicated in one of the biggest Thalassemia Centres in Europe and this is well representative of the reality of developed countries. It should not be forgotten, however, that HCV still represents a major health challenge for Thalassemia patients receiving transfusions in low-income countries, who are usually infected during their childhood [46,47].

Conflict of interest

None declared.

References

[1] Colombo M, Oldani S, Donato MF, Borzio M, Santese R, Roffi L, et al. A multicenter, prospective study of post-transfusion hepatitis in Milan. Hepatology 1987;7(4):709–12.

[2] Prati D, Zanella A, Farma E, De Mattei C, Bosoni P, Zappa M, et al. A multicenter prospective study on the risk of acquiring liver disease in anti-hepatitis C virus negative patients affected from homozygous beta-thalassemia. Blood 1998;92(9):3460–4.

[3] Angelucci E, Muretto P, Nicolucci A, Baronciani D, Erer B, Graziev J, et al. Effects of iron overload and hepatitis C virus positivity in determining progression of liver fibrosis in thalassemia following bone marrow transplantation. Blood 2002;100(1):17–21.

[4] Voskaridou E, Ladis V, Kattamis A, Hassapopoulou E, Economou M, Kourakli A, et al. A national registry of haemoglobinopathies in Greece: deducted demographics, trends in mortality and affected births. Ann Hematol 2012;91(9):1451–8.

[5] Borgna-Pignatti C, Vergine G, Lombardo T, Cappellini MD, Cianciulli P, Maggio A, et al. Hepatocellular carcinoma in the thalassaemia syndromes. Br J Haematol 2004;124(1):114–7.

[6] Borgna-Pignatti C, Garani MC, Forni GL, Cappellini MD, Cassinerio E, Fidone C, et al. Hepatocellular carcinoma in thalassaemia: an update of the Italian Registry. Br J Haematol 2014;167(1):121–6.

[7] Moukhadder HM, Halawi R, Cappellini MD, Taher AT. Hepatocellular carcinoma as an emerging morbidity in the thalassemia syndromes: a comprehensive review. Cancer 2017;123(5):751–8.

[8] Ippolito G, Capobianchi MR, Lanini S, Antonelli G. Is hepatitis C virus eradication around the corner only 25 years after its discovery? Int J Antimicrob Agents 2015;45(2):111–2.

[9] easloffice@easloffice.euEAftSotLEa. EASL recommendations on treatment of hepatitis C 2016. J Hepatol 2017;66(1):153–94.

[10] easloffice@easloffice.euEAftSotLEa, Liver EAftSot. EASL recommendations on treatment of hepatitis C 2018. J Hepatol 2018;69(2):461–511.

[11] Fernández Carrillo C, Lens S, Llop E, Pascasio JM, Crespo J, Arenas J, et al. Treatment of hepatitis C virus infection in patients with cirrhosis and predictive value of model for end-stage liver disease: analysis of data from the Hepa-C registry. Hepatology 2017;65(6):1810–22.

[12] Origa R, Ponti ML, Filosa A, Galeota Lanza A, Piga A, Saracco GM, et al. Treatment of hepatitis C virus infection with direct-acting antiviral drugs is safe and effective in patients with hemoglobinopathies. Am J Hematol 2017;92(12):1349–55.

[13] Hézode C, Colombo M, Bourlière M, Spengler U, Ben-Ari Z, Strasser SI, et al. Elbasvir/grazoprevir for patients with hepatitis C virus infection and inherited blood disorders: a phase III study. Hepatology 2017;66(3):736–45.

[14] Sinakos E, Kountouras D, Koskinas J, Zachou K, Karatapanis S, Triantos C, et al. Treatment of chronic hepatitis C with direct-acting antivirals in patients with β-thalassaemia major and advanced liver disease. Br J Haematol 2017;178(1):130–6.

[15] Mangia A, Sarli R, Gamberini R, Piga A, Cenderello G, Piazzolla V, et al. Randomised clinical trial: sofosbuvir and ledipasvir in patients with transfusion-dependent thalassaemia and HCV genotype 1 or 4 infection. Aliment Pharmacol Ther 2017;46(4):424–31.

[16] Mehta R, Kabrawala M, Nandwani S, Desai P, Bhayani V, Patel S, et al. Safety and efficacy of sofosbuvir and daclatasvir for hepatitis C virus infection in patients with β-thalassemia major. J Clin Exp Hepatol 2018;8(1):3–6.

[17] Biliotti E, Palazzo D, Serani M, Silvestri AM, Volpicelli L, Esvan R, et al. Interferon free antiviral treatment of chronic hepatitis C in patients affected by β-thalassemia major. Ann Hematol 2017;96(6):1043–5.

[18] Cappellini MD, Cohen A, Porter J, Taher A, et al., editors. Guidelines for the management of transfusion dependent thalassaemia (TDT). 3rd edition Nicosia (CY): Thalassaemia International Federation; 2014.

[19] Friedrich-Rust M, Ong MF, Martens S, Sarrazin C, Bojunga J, Zeuzem S, et al. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. Gastroenterology 2008;134(4):960–74.

[20] Wood JC, Enriquez C, Ghugre N, Tyzka JM, Carson S, Nelson MD, et al. MRI R2 and R2* mapping accurately estimates hepatic iron concentration in transfusion-dependent thalassemia and sickle cell disease patients. Blood 2005;106(4):1460–5.

[21] Borgna-Pignatti C, Rugolotto S, De Stefano P, Zhao H, Cappellini MD, Del Vecchio GC, et al. Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. Haematologica 2004;89(10):1187–93.

[22] Telfer P, Coen PG, Christou S, Hadjigavriel M, Kolnakou A, Pangalou E, et al. Survival of medically treated thalassemia patients in Cyprus. Trends and risk factors over the period 1980–2004. Haematologica 2006;91(9):1187–92.

[23] Modell B, Khan M, Darlison M, Westwood MA, Ingram D, Pennell DJ, et al. Improved survival of thalassaemia major in the UK and relation to T2* cardiovascular magnetic resonance. J Cardiovasc Magn Reson 2008;10:42.

[24] Di Marco V, Capra M, Angelucci E, Borgna-Pignatti C, Telfer P, Harmatz P, et al. Management of chronic viral hepatitis in patients with thalassemia: recommendations from an international panel. Blood 2010;116(16):2875–83.

[25] Di Marco V, D'Ambrosio R, Bronte F, Saracco G, Lanza AG, Forni G, et al. Dual therapy with peg-interferon and ribavirin in thalassemia major patients with chronic HCV infection: is there still an indication? Dig Liver Dis 2016;48(6):650–5.

[26] Di Marco V, Lo Iacono O, Capra M, Grutta S, Ciaccio C, Gerardi C, et al. Alpha interferon treatment of chronic hepatitis C in beta-thalassaemia. Gut 1993;34(2 Suppl):S142–3.

[27] Di Marco V, Bronte F, Sicily Group for the Study of Thalassemia, et al. HCV clearance among hemophiliacs and beta-thalassemics. Gastroenterology 2007;132(April (4)):1634.

[28] Alavian SM, Tabatabaei SV. Therapy of hepatitis C in thalassemia: the influence of iron on achieving sustained viral response. Ann Hematol 2010;89(2):215–6.

[29] Sievert W, Pianko S, Warner S, Bowden S, Simpson I, Bowden D, et al. Hepatic iron overload does not prevent a sustained virological response to interferon-alpha therapy: a long term follow-up study in hepatitis C-infected patients with beta thalassemia major. Am J Gastroenterol 2002;97(4):982–7.

[30] Taher AT, Musallam KM, Khalife M, Barada K. Hepatitis C antiviral response in thalassemia: what is the role of liver iron concentration? Ann Hematol 2009;88(10):1033–4.

[31] Holmes JA, Yu ML, Chung RT. Hepatitis B reactivation during or after direct acting antiviral therapy — implication for susceptible individuals. Expert Opin Drug Saf 2017;16(6):651–72.

[32] Wang C, Ji D, Chen J, Shao Q, Li B, Liu J, et al. Hepatitis due to reactivation of hepatitis B virus in endemic areas among patients with hepatitis C treated with direct-acting antiviral agents. Clin Gastroenterol Hepatol 2017;15(1):132–6.
[33] Chen G, Wang C, Chen J, Ji D, Wang Y, Wu V, et al. Hepatitis B reactivation in hepatitis B and C coinfected patients treated with antiviral agents: a systematic review and meta-analysis. Hepatology 2017;66(1):13–26.
[34] Dirchwolf M, Ruf AE. Role of systemic inflammation in cirrhosis: from pathogenesis to prognosis. World J Hepatol 2015;7(16):1974–81.
[35] Zignego AL, Giannini C, Gragnani L. HCV and lymphoproliferation. Clin Dev Immunol 2012;2012:980942.
[36] Dessì C, Clemente MG, Diana G, Congia M, Frau F, Lai ME, et al. Cryoglobulinemia in transfusion-dependent thalassemia major. Clin Exp Rheumatol 1995;(13 Suppl 13):S149–51.
[37] Emery JS, Kuczynski M, La D, Almarzooqi S, Kowgier M, Shah H, et al. Efficacy and safety of direct acting antivirals for the treatment of mixed cryoglobulinemia. Am J Gastroenterol 2017;112(8):1298–308.
[38] Bunchorntavakul C, Mitrani R, Reddy KR. Advances in HCV and cryoglobulinemic vasculitis in the era of DAAs: are we at the end of the road? J Clin Exp Hepatol 2018;8(1):81–94.
[39] Gaia S, Vanni D, Campion D, Rolle E, Stradella D, Cortegoso Valdivia P, et al. Is stiffness reliable for the detection of liver fibrosis in HCV patients treated with direct acting antiviral drugs (DAAs)? A prospective cohort study. Dig Liver Dis 2018;50(February (1)):Suppl.:36.
[40] Romano A, Angeli P, Piovesan S, Noventa F, Anastassopoulos G, Chemello L, et al. Newly diagnosed hepatocellular carcinoma in patients with advanced hepatitis C treated with DAAs: a prospective population study. J Hepatol 2018;69(2):345–52.
[41] Calvaruso V, Cabibbo G, Cacciola I, Petta S, Madonia S, Bellia A, et al. Incidence of hepatocellular carcinoma in patients with HCV-associated cirrhosis treated with direct-acting antiviral agents. Gastroenterology 2018;155(2):411–21.e4.
[42] Roche B, Coilly A, Duclos-Vallee JC, Samuel D. The impact of treatment of hepatitis C with DAAs on the occurrence of HCC. Liver Int 2018;38(Suppl 1):139–45.
[43] Coates TD, Carson S, Wood JC, Berdoukas V. Management of iron overload in hemoglobinopathies: what is the appropriate target iron level? Ann N Y Acad Sci 2016;1368(1):95–106.
[44] Uchino K, Tateishi R, Fujiwara N, Minami T, Sato M, Enooku K, et al. Impact of serum ferritin level on hepatocarcinogenesis in chronic hepatitis C patients. Hepatol Res 2016;46(4):259–68.
[45] Nirei K, Matsuoka S, Nakamura H, Matsumura H, Moriyama M, et al. Incidence of hepatocellular carcinoma reduced by phlebotomy treatment in patients with chronic hepatitis C. Intern Med 2015;54(2):107–17.
[46] Atwa ZT, Abdel Wahed WY. Transfusion transmitted infections in frequently transfused thalassemic children living in Fayoum Governorate, Egypt: current prevalence and risk factors. J Infect Public Health 2017;10(6):870–4.
[47] Sultan S, Irfan SM, Siddiqui M, Zaidi SM. Current trends of seroprevalence of transfusion transmitted infections in Pakistani β-thalassaemic patients. Malays J Pathol 2016;38(3):251–5.