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Efficacy evaluation of new anti-HCV treatments after a two-year use

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ABSTRACT:

— ***Background:*** The hepatitis C virus (HCV) is currently present worldwide and is the principal cause of chronic hepatitis C and hepatocellular carcinoma, thus representing one of the most important public health concern. The most advanced knowledge of the replicative cycle of this virus has permitted the identification of certain biological targets for which drugs with a direct antiviral action have been developed (DAA).

— ***Patients and Methods:*** In the present study, we present the results obtained in the first 24 months of use of these new drugs in our Clinic. Data regarding the first 360 treatments delivered between January 2015 and December 2016 were analyzed. A quantitative dosage of HCV-RNA was evaluated 12 weeks after the end of therapy.

— ***Results:*** A sustained virological response at week 12 (SVR12) was noted in 346 cases (96.1%), 13 of which (3.6%) demonstrated a relapse within 12 weeks at the end of therapy (relapsers), and in one case (0.3%) there was no response (non-responder).

— ***Conclusions:*** The triple therapy was shown to be highly potent even in the most difficult patients, with a negative viremia within 4 weeks from initiation. Follow-up studies of patients with advanced cirrhosis (Child A and B) are necessary to evaluate the long-term efficacy of the therapy and the impact on the difficult complications of the advanced liver disease. A more extensive use of resistance testing before initiating treatment might be considered, especially in patients with an extensive pre-DAA therapy history.

— ***Keywords:*** HCV, Chronic hepatitis, Hepatocellular carcinoma, DAA, Direct antiviral action, SVR, Sustained response, Resistance testing.

INTRODUCTION

HCV has been shown to be the main worldwide cause of chronic hepatic disease[1]. At present, in industrialized countries, it is responsible for 10% of acute hepatitis, and more than 50% of chronic hepatitis, 27% of cirrhosis and 25% of hepatocellular carcinomas[2]. Thus representing one of the most important public health problems.

The prevalence of HCV infection in Italy is more than 3% with greater values for elderly patients (30% of the elderly population in regions of Southern Italy) demonstrating a rather diversified geographical distribution.The evidence: a higher frequency in Central Italy (8%) and in the South (12-16%) compared to 2-3% in Northern Italy[3]. The actual estimate of 150-180 million chronic carriers worldwide (in particular, 1.5 million in Italy and 5-10 million in Europe) leads to the prevision of a significant increase in hepatic complications (decompensated cirrhosis, hepatocarcinoma) in the next 10-20 years[4].

However, in recent years great progress has been made in the treatment of patients with hepatitis C. Up to a few years ago, the therapeutic strategy utilized pegylated interferon (PEG-IFN alfa 2a or 2b) plus ribavirin (RBV)[5]. The progressive knowledge of the HCV replicative cycle has permitted the identification of biological targets for which drugs with a direct-

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acting antiviral effect (first generation DAA) have been developed. The first among these, boceprevir (BOC) and telaprevir (TVR), non-structural serine-protease inhibitors (NS3/4) of HCV. For these, the Italian Agency of Drugs (AIFA) permitted reimbursement[6]. In spite of evident improvement, these drugs were the cause of serious collateral effects: complex posologic schemes of extensive duration, and a low percentage of sustained virological response (SVR) in cirrhotic patients and those non-responsive to interferon (null responders)[7-10]. However, the real revolution in the field of therapy for chronic hepatitis C was obtained about 6 months later, from November 12, 2014 (data in which the AIFA permitted reimbursement for sofosbuvir[11]) to May 18, 2015 (when reimbursement was permitted for the last two antiviral molecules placed in commerce in Italy: ombitasvir/paritaprevir/ritonavir and dasabuvir)[12].

In this study, we have analyzed data regarding the first 24 months of use of the new DAA c/o the Infective Disease Unit of the Bisceglie hospital. It must be pointed out that in Italy, at present, only Sofosburvir (Sovaldi – SOF) alone or in association with ledipasvir (Harvoni – LDV/SOF), simeprevir (Olysio – SMV), daclatasvir (Daklinza – DVC) and dasabuvir (Exviera – DSV) are commercially available. The association of paritaprevir/ombitas/ritonavir (Viekirax – PV/r/OBV) is also utilized.

PATIENTS AND METHODS

From January 2015 and December 2016, 360 treatments were analyzed using both the date of HCV/RNA after at least 12 weeks and after the end of treatment. The 360 DAA treatments were prescribed in accordance with the seven priority criteria established by the Italian Drug Agency (AIFA)[13]. A document was addressed to the Italian Association for Study of the Liver (AISF) for the rational use of this drug, published December 14 2016 online and periodically updated during the course of time)[14]. A quantitative dosage of HCV-RNA was acquired for all patients between the 29th and 38th day of therapy (as indicated by AIFA) and after 12 or eventually 24 weeks of therapy, and at 12 weeks after the end of therapy.

The 360 treatments were distributed to 355 patients (5 patients were subjected to more than one treatment, 2 patients died of nonrelated causes). We did not take in account therapies still ongoing or completed but results of quantitative HCV-RNA not available. The treatments (only one), in which the patient was viremic before the end of therapy were interrupted, but obviously considered. In two cases the patient died during treatment, but in neither of these cases was death correlated to treatment; however, these patients were not included.

For the most part, the patients were males (55.5%), medium age was 62.5 years (range 22-83), and only five patients were not Italian. A total of 64.5% of patients (227) were affected with HCV genotype 1 (84.5% of these were genotype 1b), 21% with genotype 2, 10% with genotype 3 and 5% with genotype 4). All non-cirrhotic patients underwent biopsy, and, if not possible, to hepatic elastometry. If hepatic cirrhosis had already been clinically diagnosed, it was not necessary to perform further tests to evaluate the hepatic fibrosis, as determined by AIFA criteria. Only 2% (7 patients) had a fibrosis grade F0-F2, 167 (47%) had grade F3, and the remaining 51% (181 patients) had grade F4 (according to the METAVIR score or

Table 1. Clinical characteristics of patients treated with DAA and type of treatments prescribed.

Patients	**No. 355**
Male	200 (55.5)
Average age	62.5 (22-83)
HCV genotype	**No. (%)**
1	27 (7.6)
1a	8 (2,3)
1b	192 (54)
2	5 (1,4)
2a/2c	70 (19,7)
3	35 (9,9)
4	18 (5,1)
Fibrosis	**No. (%)**
F0-F2	7 (2)
F3	167 (47)
F4 o cirrhosis	181 (51)
Co-morbility	**No. (%)**
HIV	35 (9.8)
Cryoglobulinemia	10 (2.8)
History of hemopathia	2 (0.5)
History of HCC	5 (1.4)
Liver transplant	3 (0.8)
Kidney transplant	1 (0.2)
Treatments	**No. (%)**
Provided	360
Completed	359 (99.7)
Suspended	1 (0.3)
Patients with SVR12	347 (96.1)
Prescribed regimens	**No. (%)**
Sofosbuvir	79 (22)
Sofosbuvir/simeprevir	61 (17)
Sofosbuvir/ledipasvir	158 (44)
Sofosbuvir/daclatasvir	40 (11)
Ombitasvir/paritaprevir/ritonavir	7 (2)
Ombitasvir/paritaprevir/ritonavir/dasabuvir	13 (3.6)
Sofosbuvir/Peg-IFN	1 (0.4)
Simeprevir/Peg-IFN	1 (0.4)
Weeks of treatment	**No. (%)**
12	115 (32)
16	1 (0,2)
24	244 (67.8)
Use of Ribavirin	**no. (%)**
	115 (32)

Table 2. Treatments provided, completed and failures.

Prescribed regimens	Provided No./%	Completed No./%	Failed No./(%)
Sofosbuvir	79 (22)	79 (100)	8 (10.1)
Sofosbuvir/Simeprevir	61 (17)	61 (100)	3 (4.9)
Sofosbuvir/Ledipasvir	158 (44)	157 (99.4)	1 (0,6)
Sofosbuvir/Daclatasvir	40 (16)	40 (100)	1 (2.5)
Ombitasvir/Paritaprevir/Rtv	7 (2)	7 (100)	0
Ombitasvir/Paritaprevir/Rtv/Dasabuvir	13 (3.6)	13 (76.9)	0
Sofosbuvir/Peg-IFN	1 (0.2)	1 (100)	1 (100)
Simeprevir/Peg-IFN	1 (0.2)	1 (100)	0
Total	360	359 (99.7)	14 (3.8)

corresponding ISHAK score) or had clinical cirrhosis. Among the 355 patients, there were 35 patients with HIV infection, 10 with cryoglobulinemia, 2 had been administered chemotherapy in the past for hematologic neoplasm, and lastly, 4 were treated for solid organ transplantation (3 for liver and 1 for kidney). The DAA used were LDV/SOF (158 treatments 44%), followed by SOF (22%), SOF+SMV (17%), SOF+DVC (11%), and PV/r/OBV with or without DSV (5.6%). The duration of the treatment was 24 weeks in 2/3 of patients (68%); in 32% of cases, ribavirin was added to the direct-acting antiviral therapy.

Resistance testing was carried out only in pre-therapy selected cases and in the single non-responder patient at the moment of failure with the sofobuvir/ledipasvir regimen. HCV-RNA was extracted using a commercial kit (Qiagen, Hilden, Germany). The protocol with retrotranscription (RT-PCR) was carried out with a set of home-made primers for the NS5B, NS5A, NS3 regions; the kit for sequencing the ABI *prism BigDye* (Applied Biosystems, Foster City, CA, USA) was utilized for sequencing the amplification products in both helices using the ABI 3130 analyser (Applied Biosystems). Lastly, the sequences were analyzed with Seqscape v2.5 software (Applied Biosystems, Foster City, CA, USA). For the interpretation of sequences, the software geno2pheno for HCV, which is a system of web-based interpretation[15] available free-of-charge *online*[16], was utilized.

Table 1 illustrates the main anagraphic and clinical data of the patients for whom a therapy with at least one direct-action drug was prescribed.

RESULTS

We herein present the results pertaining to our first 360 therapeutic regimens prescribed and completed, and for whom at least a 12-week follow-up is available; the viremia after the first four weeks of therapy was also available.

All patients were negative (HCV-RNA <15 or 12 IU/ml according to the commercial kit utilized) after the first four weeks of therapy.

Of the 360 treatments, a SVR 12 was reached in 347 (96.1%) cases. Table 2 summarizes the treatments utilized, those terminated and those non-resolved. A total of 14 patients did not reach a SVR: 9 of whom failed a regimen composed of SOF (in 8 cases with ribavirin, and 1 case with ribavirin and pegylated interferon); 3 failed a regimen composed of SOF+SMV (all with genotype 1); 1 patient with genotype 3a failed a regimen composed of SOF+DCV, and 1 patient with genotype1b did not respond to LDV/SOF. The 14 patients (8 males) were all cirrhotic, with a median age of 58.7 years; the duration of therapy was 24 weeks for 10/14 cases and in 13 cases it was associated with ribavirin.

In Table 3, the characteristics of the 14 patients who did not reach a SVR12 are summarized: 5 of the

Table 3. Characteristics of the 14 patients who did not reach a SVR12.

No SVR12	Cirrhosis	Genotype	Treatment	RBV	Weeks	Co-morb.
Relapser	Yes	3a	SOF	Yes	24 w	
Relapser	Yes	2a/2c	SOF	Yes	24 w	
Relapser	Yes	2a/2c	SOF	Yes	24 w	
Relapser	Yes	2a/2c	SOF	Yes	24 w	
Relapser	Yes	2a/2c	SOF	Yes	24 w	
Relapser	Yes	2a/2c	SOF	Yes	24 w	HCC
Relapser	Yes	2a/2c	SOF	Yes	24 w	LNH
Relapser	Yes	2a/2c	SOF	Yes	24 w	
Relapser	Yes	3a	SOF+PEG-IFN	Yes	12 w	
Relapser	Yes	1a	SOF+SMV	No	12 w	
Relapser	Yes	1b	SOF+SMV	Yes	12 w	
Relapser	Yes	1b	SOF+SMV	Yes	24 w	HCC
Relapser	**Yes**	**3a**	**SOF+DCV**	**Yes**	24 w	HIV+
Non responder	**Yes**	**1b**	**LDV/SOF**	**Yes**	12 w	

8 patients who relapsed after treatment with SOF+RBV already initiated a new treatment with the regimen currently prescribed in these cases (SOF+DCV).

Of the 14 patients failing, 2 had a history of hepatic carcinoma, 1 had a history of a previous diagnosis of B-cell lymphoma and one was HIV-infected. Moreover, the non-compliant referral of the patient who failed the SOF+DCV regime and genotype 3a must be noted.

Figure 1 compares the results of the resistance test of the NS5A protein of the polymerase of the patient failing administration of Harvoni, carried out with the geno2pheno® software, which demonstrates the 31M and the 93H mutations responsible for the extensive class resistance. Moreover, the resistance testing for both the NS5B protein of the polymerase and the NS3 protein of the protease was carried out but did not show mutations responsible for pharmacoresistance to the other drug classes.

Two deaths occurred, not directly correlated with therapy; the first patient, a male of 65 years previously subjected to liver and kidney transplant and treated with immunosuppressive therapy, died after terminating treatment with LDF/SOF for acute hepatic insufficiency (without completing the 12 weeks after end of treatment); the second case regarded a woman 64 years old who died after eight weeks of treatment for a post-traumatic cerebral hemorrhage.

DISCUSSION

The new direct-acting antivirals are demonstrating, as expected, to be extremely effective in our case studies; SVR was reached in more than 96% of patients. Of the 14 patients who failed, 10 were treated with schemes which after a few months were considered "suboptimal" or, in any case, would not be used today: sofosbuvir without daclatasvir for cirrhotic patients with genotype 2 (8 cases) or with genotype 3 (1 case) and simeprevir for genotype 1a. In the 3 resistant patients, 2 were genotype 1b with a cirrhotic hepatitis treated with sofosbuvir/simeprevir, and the last patient was a genotype 3 co-infected with HIV who did not correctly take the therapy.

Certain evident differences regarding the type of regimen prescribed depend on when the drug was placed on the pharmaceutical market. Treatment with SOF and SOF+SMV has a long prescription history as it has been commercially available for longer. Consequently, as it became possible to prescribe other drugs and associations of more molecules in a single capsule, the use of this regimen has diminished. Moreover, the indications for treatment have changed. The most evident example is the possibility to treat genotype 2 with the association of SOF+DCV compared to prescribing only SOF, as previously noted.

Even though our results are extremely positive, it must be emphasized that in the group of patients that did not reach a sustained response to therapy, the comorbidities (at least those considered most important, such as HIV, HCV-correlated compliance) were found in a percentage which was double that of responders. In fact 28% of the 14 patients who failed presented comorbidities compared to the 14% of those who were successfully treated. The HIV seropositivity, however, appears not to have had an important role and did not influence the response to therapy in our patients. Moreover, resistance testing in our opinion is useful in selected cases and is certainly crucial in patients with previous treatment failure.

CONCLUSIONS

The new DAA, characterized by a low pill burden, have greatly improved the outcomes of the first-generation DAA in terms of efficacy, tolerability.

However, since few studies on long-term follow-up are currently available, it is important to collect prospective *real-life* data on the survival of patients with cirrhosis in whom the viral clearance has been obtained.

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Conflicts of Interest (COI):
No potential conflict of interest relevant to this article exists.

NS5A codons covered	19 - 213
NS5A region (w.r.t. D90208)	L31M, L34V, Q54H, T83M, Y93H, V138I, V164A, E171D, V174T, V196T, A197T
NS5A region (w.r.t. H77)	K24Q, A25S, M28L, Q30R, L31M, I34V, V37L, R44K, R56T, H58P, E62Q, T71S, R78K, R81S, S85H, Y93H, L101S, K107S, F108R, S114A, I121V, R122T, S131T, L138I, I144V, S146A, L153V, F161Y, P164A, E171D, S174T, R176Q, H180N, E181Q, P183L, V196T, A197T, A213T

Drug	Prediction		Scored Mutations
Daclatasvir	resistant		31M,93H
Elbasvir	resistant		31M,93H
Ledipasvir	resistant		31M,93H
Ombitasvir	resistant		31M,93H

Figure 1. NS5A protein of the polymerase resistance test of the patient failing administration of Harvoni, (geno2pheno® software).

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