May some HCV genotype 1 patients still benefit from dual therapy? The role of very early HCV kinetics

Monica Tontodonati[1-2], Valeria Cento[3], Ennio Polilli[4], Cecilia Colabattista[1], Raffaella Cascella[5], Maria Pina Sciotti[6], Dante Di Giammartino[7], Francesca Trave[1], Velia Chiara Di Maio[3], Roberto Monarca[8], Francesco Di Candilo[9], Roberta Prinapori[10], Elena Rastrelli[8], Jacopo Vecchiet[2], Francesca Ceccherini-Silberstein[3], Lamberto Manzoli[11], Emiliano Giardina[5-12], Carlo Federico Perno[3], Giustino Parruti[1]

[1]Infectious Disease Unit, Pescara General Hospital, Pescara, Italy;
[2]Clinic of Infectious Diseases, Department of Medicine and Science of Aging, University "G. d'Annunzio" Chieti-Pescara, Chieti, Italy;
[3]Virology Unit, Department of Experimental Medicine and Surgery, "Tor Vergata" University of Rome, Rome, Italy;
[4]Microbiology and Virology Unit, Pescara General Hospital, Pescara, Italy;
[5]Department of Biomedicine and Prevention, School of Medicine, University of Rome "Tor Vergata", Rome, Italy;
[6]Infectious Disease Unit, Vasto General Hospital, Vasto (CH), Italy;
[7]Infectious Disease Unit, "G. Mazzini" Teramo General Hospital, Teramo, Italy;
[8]Infectious Disease Unit, Belcolle General Hospital, Viterbo, Italy;
[9]Clinic of Infectious Diseases, "Santa Maria della Misericordia" Hospital, Perugia, Italy;
[10]Infectious Disease Unit, IRCCS San Martino Hospital-IST, Genoa, Italy;
[11]Section of Hygiene, Epidemiology, Pharmacology and Legal Medicine, University of Chieti-Pescara, Chieti, Italy;
[12]Molecular Genetics Laboratory UILDM, Santa Lucia Foundation, Rome, Italy

SUMMARY

When treating HCV patients with conventional dual therapy in the current context of rapidly evolving HCV therapy, outcome prediction is crucial and HCV kinetics, as early as 48 hours after the start of treatment, may play a major role. We aimed at clarifying the role of HCV very early kinetics.

We consecutively enrolled mono-infected HCV patients at 7 treatment sites in Central Italy and evaluated the predictive value of logarithmic decay of HCV RNA 48 hours after the start of dual therapy (Delta48).

Among the 171 enrolled patients, 144 were evaluable for early and sustained virological response (EVR, SVR) prediction; 108 (75.0%) reached EVR and 84 (58.3%) reached SVR. Mean Delta48 was 1.68±1.22 $\log_{10}$ IU/ml, being higher in patients with SVR and EVR.

Those genotype-1 patients experiencing a Delta48 >2 logs showed a very high chance of success (100% positive predictive value), even in the absence of rapid virological response (RVR).

Evaluation of very early HCV kinetics helped identify a small but significant proportion of genotype-1 patients (close to 10%) in addition to those identified with RVR, who could be treated with dual therapy in spite of not reaching RVR.

In the current European context, whereby sustainability of HCV therapy is a crucial issue, conventional dual therapy may still play a reasonable role in patients with good tolerance and early prediction of success.

KEY WORDS: HCV dual therapy, Early viral kinetics, HCV therapy optimization, EVR, RVR.

Received December 12, 2014 *Accepted* September 11, 2015

Corresponding author
Monica Tontodonati, MD
Infectious Disease Unit
Pescara General Hospital
Via Fonte Romana, 8 - 65124 Pescara, Italy
E-mail: monicatontodonati@gmail.com

INTRODUCTION

HCV therapy is rapidly evolving, depicting HCV eradication as feasible for most patients with triple interferon (IFN)-based therapies and IFN-free regimens (Hoofnagle and Sherker, 2014; Asselah and Marcellin, 2014; Muir, 2014). The

latter, however, are not yet available in Europe or in low-income countries. The higher costs of these new regimens and financial constraints in health systems all around the world, especially in countries with a high HCV burden (Bruggman *et al.,* 2014) make therapy optimization mandatory for HCV treatment sustainability. Moreover higher side effects with triple IFN-based therapy (Chopra *et al.,* 2013), the current alternative to conventional dual therapy, have to be considered. Indeed, a fair proportion of HCV patients, up to 50%, can still be treated with conventional dual therapy with PEG-IFNα and ribavirin (RBV) (Durante-Mangoni *et al.,* 2009; Parruti *et al.,* 2010; Marcellin *et al.,* 2012; Andriulli *et al.,* 2014), with limited costs and well-known side effects. Moreover, most of recently released HCV guidelines around Europe (EASL, 2014; AISF, 2014) suggest starting with conventional dual therapy in all genotype-1 naïve patients without liver fibrosis, to spare third drug toxicities when rapid virological response (RVR) is achieved. Accurate prediction of HCV treatment success may therefore be more relevant now in the era of therapy personalization, to allocate the right patient to the right treatment, keeping high chances of success for everyone and addressing newer therapeutic approaches to those patients with low chances of success with conventional treatment (EASL, 2014; AISF, 2014). The importance of baseline predictors is crucial (Beinhardt *et al.,* 2013; Bruggman *et al.*), but on-treatment predictors, that is early viral kinetics, play the main role being a very good proxy of treatment outcome (Durante-Mangoni *et al.,* 2009; Parruti *et al.,* 2010; Andriulli *et al.,* 2014). A recent study from Andriulli *et al.* (2014) showed that combined evaluation of baseline predictors for sustained virological response (SVR) and viral kinetics (namely RVR) will identify a large proportion of patients with >80% chance of cure with dual therapy alone, as also highlighted in the Prophesys cohort (Marcellin *et al.,* 2012). Evaluation of earlier viral kinetics, up to as early as 48 hours after the start of dual therapy, revealed a good predictive value both for early virological response (EVR) and SVR in 2 monocentric Italian cohorts (Durante-Mangoni *et al.,* 2009; Parruti *et al.,* 2010).

We set up a prospective multicentric evaluation of HCV RNA decay 48 hours after the start of dual therapy, to clarify the role of very early HCV RNA kinetics in predicting treatment outcome.

MATERIALS AND METHODS

A multicentric cohort of consecutive HCV mono-infected patients treated with PEG-IFNα and RBV was enrolled in 2011-2012 at 7 sites of care, mostly based in central Italy. All patients provided written consent. The study was approved by ethics committees at study sites in Pescara, Chieti, Teramo, Viterbo and Perugia, and it was conducted according to provisions of the Declaration of Helsinki and Good Clinical Practice Guidelines. Demographic, clinical and virological characteristics of patients were collected at therapy start (Table 1). Cirrhosis was diagnosed according to Metavir score on liver biopsy (Bedossa and Poynard, 1996) or on the occurrence of one or more following criteria: a liver elastometric value ≥13 KPa (Fibroscan®), and/or oesophageal varices at endoscopy, and/or platelet count <100,000/mL-1 (Symonosky, 1999; Castera, 2012; Poynard *et al.,* 2012; Andriulli *et al.,* 2014). Autoimmune diathesis, thyroid autoimmunity, depressive disorders, diabetes and other relevant co-morbidities (previous cancer, substitution opioid therapy, or other chronic diseases) were recorded.

Patients were screened for two SNPs of the *IL28B* gene (rs12979860 T/C, rs8099917 T/G) in a centralized reference laboratory. Genomic DNA was extracted from buccal swabs or peripheral blood using standard procedures. *IL28B* genotyping analysis was performed by Real Time-PCR using TaqMan® chemistry. The AB7500 Fast real-time PCR instrument and the Sequence Detection System 2.1 software (Applied Biosystems, Foster City, California) were used for genotyping. Each plate contained a negative control sample and three positive control samples previously confirmed by direct sequencing.

Patients received either PEG-IFNα-2a or 2b plus weight-based RBV. Plasma HCV-RNA was quantified by Roche COBAS® TaqMan® HCV Test v2.0 (LLOQ, lower limit of quantification: 25 IU/mL-1; LLOD, lower limit of detection: 15 IU/ml-1) at baseline, at very early time-points

TABLE 1 - *Characteristics of the evaluable patients.*

	Total sample (144 pts)	*Genotype 1 (81 pts)*	*Genotype 2 (30 pts)*	*Genotype 3 (33 pts)*	*p-value**
Male gender, n (%)	88 (61.1)	50 (61.7)	9 (30.0)	28 (84.8)	<0.001
Age, y (SD)	45.8 (13.7)	47.4 (13.9)	51.1 (15.3)	39.0 (9.4)	0.0003
Cirrhosis, n (%)	31 (21.5)	21 (25.9)	4 (13.3)	7 (21.2)	0.45
Baseline HCV RNA ≥400.000 UI/ml, n (%)	99 (68.7)	55 (67.9)	21 (70.0)	23 (69.7)	0.97
Altered ALT, n (%)	88 (63.7)	51 (63.8)	11 (44.0)	26 (78.8)	0.024
rs12979860 CC[a], n (%)	24 (34.8)	15 (41.7)	4 (30.8)	5 (25.0)	0.43
rs8099917 TT[b], n (%)	37 (63.8)	16 (59.3)	7 (58.3)	14 (73.7)	0.55
Thyroid autoimmunity, n (%)	6 (4.3)	4 (4.9)	2 (6.7)	0 (0.0)	0.38
Autoimmune diathesis, n (%)	19 (13.4)	10 (12.5)	4 (13.3)	5 (15.6)	0.91
Depression, n (%)	11 (7.8)	8 (10.1)	2 (6.7)	1 (3.0)	0.43
Diabetes, n (%)	9 (6.3)	4 (4.9)	2 (6.7)	3 (9.4)	0.68
Other comorbidities[c], n (%)	58 (43.9)	31 (40.8)	11 (39.3)	16 (51.6)	0.54
Naïve patients, n (%)	127 (90.1)	71 (89.9)	27 (93.1)	29 (87.9)	0.79
PEG IFNα-2a, n (%)	79 (57.3)	56 (71.8)	7 (24.1)	16 (51.6)	<0.001
Delta48, log10 IU/ml (SD)	1.67 (1.21)	1.10 (0.93)	2.51 (0.85)	2.28 (1.33)	0.001
RVR, n (%)	69 (47.3)	17 (21.0)	28 (93.3)	22 (66.7)	<0.001
EVR, n (%)	107 (76.9)	47 (59.5)	30 (100.0)	30 (90.9)	<0.001
SVR, n (%)	84 (58.3)	38 (46.9)	23 (76.7)	22 (66.7)	0.020

*Chi-squared test for categorical variables, Kruskal-Wallis test for continuous variables. [a]Evaluable for 69 patients only (36 genotype 1, 13 genotype 2, 20 genotype 3); [b]evaluable for 58 patients only (27 genotype 1, 12 genotype 2, 19 genotype 3); [c]previous cancer, substitution opioid therapy, or other chronic diseases.

after therapy start (after 48 h) and at 4-12-24-48-72 weeks. Paired samples at baseline and after exactly 48h since treatment start were collected for each patient and processed in a centralized reference laboratory in the same analytic session, to minimize the potential influence of inter-assay variation (Parruti *et al.,* 2010).

Treatment was managed according to European guidelines as in 2011 (EASL, 2011). Discontinuations for both adverse effects and virological non response were recorded. The logarithmic difference between baseline and 48h HCV RNA (Delta48) (Parruti *et al.,* 2010) was evaluated in several logarithmic cut-offs both in the total sample and for genotype-1 (G1) patients; sensitivity, specificity, positive and negative predictive values (PPVs and NPVs) for each of them were calculated. SVR was defined as negative HCV RNA 24 weeks after treatment completion. Statistical significance was defined as a two-sided P-value <0.05 for all analyses; multiple regression models were fit using Stata version 10.1 (Stata Corp., College Station, TX, USA, 2010).

RESULTS

One hundred and seventy-five Caucasian HCV patients starting dual therapy were consecutively enrolled in 2011-2013; 21 patients did not show up at 48 h. One hundred and fifty-four patients were therefore evaluable. Among them, 61.1% were males, mean age was 45.8±13.7 years, 56.3% were HCV G1, 23.0% were cirrhotics. The majority of patients (90.1%) were naïve to antiviral treatment. Major co-morbidities were present in 43.9% of patients. Autoimmunity diathesis, thyroid autoimmunity, depression and diabetes, however, were not frequent (less than 10% of patients). IL28b genotypes were evaluated in a third of patients only (Table 1). Baseline characteristics of patients are summarized in Table 1. Baseline HCV viremia was ≥400.000 UI/ml-1 in 67.3% of patients. Mean Delta48 was 1.68±1.22 $\log_{10}$ IU/ml-1. Delta48 was significantly higher in non-G1 patients (2.38±1.31 vs 1.10±0.93 $\log_{10}$ IU/ml, p<0.001), in patients treated with PEG-IFNα2b (2.08±1.10 vs 1.41±1.23, p<0.001) and non cirrhotics (1.79±1.26 vs 1.12±1.02 $\log_{10}$ IU/

ml, p=0.013); moreover, Delta48 was significantly higher in patients with EVR (1.98±1.11 vs 0.78±0.81 log_{10} IU/ml, p<0.001) and SVR (1.97±1.16 vs 1.19±1.06 log_{10} IU/ml, p<0.001), showing no significant association with age, diabetes or other co-morbidities.

TABLE 2 - *Univariate analyses for EVR.*

	EVR Total sample	*p-value**	*EVR in G1 patients*	*p-value**
Gender, %		0.3		
- Female	72.4			
- Male	79.6			
Age, y (SD)		**0.030**		0.064
- <50 years	82.3		68.8	
- >50 years	66.7		48.6	
Cirrhosis, %		**<0.001**		**<0.001**
- No	85.1		75.0	
- Yes	45.8		18.8	
Baseline HCV RNA, IU/ml		0.2		0.061
<400,000	83.7		75.0	
≥400,000	75.6		53.7	
ALT, IU/ml (SD)		0.2		0.081
- Normal	82.4		73.3	
- Upper Normal Level	72.8		53.9	
rs12979860 CC, %		0.2		**0.040**
- No	75.0		50.0	
- Yes	88.9		82.4	
rs8099917 TT, %		**0.004**		**0.013**
- No	61.9		36.4	
- Yes	92.3		82.4	
Thyroid pathology, %		0.9		0.7
- No	76.8		61.2	
- Yes	75.0		50.0	
Autoimmune diseases, %		0.7		0.9
- No	76.3		60.8	
- Yes	80.0		60.0	
Depression, %		0.4		0.3
- No	75.7		58.1	
- Yes	85.7		77.8	
Diabetes, %		0.5		0.1
- No	77.3		62.5	
- Yes	66.7		25.0	
Other comorbidities, %		0.1		0.2
- No	71.3		53.3	
- Yes	82.8		68.6	
Previous treatments, %		0.3		0.3
- No	77.6		62.2	
- Yes	66.7		44.4	
PEG IFN, %		0.4		0.4
- PEG IFNα-2a	73.2		62.7	
- PEG IFNα-2b	79.7		52.2	
Delta48, log_{10} UI/ml (SD)		**<0.001**		**0.001**
≤0.5 log_{10}	39.1		26.3	
>0.5≤1 log_{10}	58.6		56.5	
>1≤1.5 log_{10}	68.8		61.5	
>1.5<2 log_{10}	89.5		81.8	
≥2 log_{10}	100.0		100.0	
RVR, %		**<0.001**		**<0.001**
- No	55.6		50.0	
- Yes	100.0		100.0	

*Chi-squared test for categorical variables, t-test for continuous variables.

Among the 154 evaluable patients, 10 stopped treatment before 12 weeks due to side effects and were not evaluable for EVR in our analysis. Among the others (144), 69 patients (47.3%) reached RVR, 108 (75.0%) reached EVR and 84 (58.3%) reached SVR. Among the 77 patients without RVR, EVR was reached by 85.7% (10/12) of non-G1 patients, and by 50.0% (33/66) of G1 patients. Among all G1 patients evaluable, EVR was significantly associated with several factors, as described in Table 2. In particular, Delta48 was 1.98±1.11 log_{10} IU/ml in patients with EVR and 0.78±0.81 log_{10} IU/ml in patients without EVR ($p<0.001$), suggesting a strong prediction potential. Therefore we tested sensitivity, specificity, PPV and NPV for Delta48 different cut-offs for the prediction of EVR, as shown in Table 3a. Interestingly, Delta48 >2 log showed a 100% PPV for EVR, whereas Delta48 >1.5 log showed a PPV as high as 91.3%. Similar results were obtained considering patients without RVR alone (Table 3b). Among them, the Delta48 2-log cut-off identified 3 patients (7.85% of G1 patients) without RVR who later reached EVR; the 1.5-log cut-off identifies 8 patients (10.3% of G1 patients). Separate evaluation for cirrhotic and non cirrhotic patients led to the same results (data not shown). Multivariate analyses confirmed the association of Delta48 with EVR for all the investigated cut-offs (2-log, 1.5-log, 1-log,0.5-log; data not shown).

DISCUSSION

Prediction of treatment success with conventional dual therapy is crucial in the current context of newer HCV drugs and regimens, where rate of success, costs and side effects have to be accurately balanced (Chopra, 2013; Hoofnagle, 2014; Muir, 2014). Indeed, the mere possibility of a long-term sustainabili-

TABLE 3 - *Prediction of EVR on the basis of delta48 in genotype 1 patients [a] and in patients without RVR [b].*

A) Patients with HCV genotype 1 (78 patients).

Delta48 cut-off	*EVR n (%)*	*Non EVR n (%)*	*p[a]*	*Sens[b] (%)*	*Spec[b] (%)*	*PPV (%)*	*NPV (%)*
2 log_{10}			0.004	26.2	100.0	**100.0**	53.7
>2 log_{10}	11 (100.0)	0 (0)					
≤2 log_{10}	31 (46.3)	36 (53.7)					
1.5 log_{10}			<0.001	44.7	93.5	91.3	52.7
>1.5 log_{10}	21 (91.3)	2 (8.7)					
≤1.5 log_{10}	26 (47.3)	29 (52.7)					
1 log_{10}			0.001	61.7	77.4	80.6	57.1
>1 log_{10}	29 (80.6)	7 (19.4)					
≤1 log_{10}	18 (42.9)	24 (57.1)					
0.5 log_{10}			0.001	89.4	45.2	71.2	73.7
>0.5 log_{10}	42 (71.2)	17 (28.8)					
≤0.5 log_{10}	5 (26.3)	14 (73.7)					

B) Patients with HCV genotype 1 without RVR only (61 patients).

Delta48 cut-off	*EVR n (%)*	*Non EVR n (%)*	*p[a]*	*Sens[b] (%)*	*Spec[b] (%)*	*PPV (%)*	*NPV (%)*
2 log_{10}			0.071	10.0	100.0	**100.0**	53.4
>2 log_{10}	3 (100.0)	0 (0)					
≤2 log_{10}	27 (46.6)	31 (53.4)					
1.5 log_{10}			0.033	26.7	93.5	80.0	56.7
>1.5 log_{10}	8 (80.0)	2 (20.0)					
≤1.5 log_{10}	22 (43.1)	29 (56.9)					
1 log_{10}			0.013	53.3	77.4	69.6	63.2
>1 log_{10}	16 (69.6)	7 (30.4)					
≤1 log_{10}	14 (36.8)	24 (63.2)					
0.5 log_{10}			0.006	86.7	45.2	60.5	77.8
>0.5 log_{10}	26 (60.5)	17 (39.5)					
≤0.5 log_{10}	4 (22.2)	14 (77.8)					

[a]chi-squared test. [b]Sens: sensitivity, Spec: specificity.

ty of IFN-based therapy will likely rest upon the possibility of a very accurate prediction of therapeutic success using relatively short IFN-based protocols, little used in clinical practice so far (Mangia, 2014). This prospective study on early HCV kinetics confirms the predictive value of Delta48 for EVR and SVR in a multicentric cohort of HCV patients enrolled in Central Italy. In particular, the 2-log cut-off showed a surprisingly high PPV (100%) for EVR in G1 patients, both with and without RVR, identifying a small but significant subset of patients with a perfect prediction of success already 48 hours after treatment start. Early viral kinetics, in addition to well-established 1- and 3-month evaluation after treatment start, have already been investigated as predictors of treatment response in recent years (Durante-Mangoni *et al.*, 2009; Parruti *et al.,* 2010): Durante-Mangoni *et al.* (2009) first showed a good NPV for SVR for a 0.5-log decay of HCV RNA 48 hours after therapy start in a monocentric cohort of Italian HCV patients (Durante-Mangoni *et al.,* 2009). These data were confirmed in another Italian monocentric evaluation by our group (Parruti *et al.,* 2010), showing a good NPV (95%) also for EVR. The current study clarifies the predictive role of Delta48 in mono-infected patients, indicating that those patients with >2 log decay at 48h have a very high chance of EVR even when RVR is not achieved and, therefore, a 95% chance of SVR (Hoofnagle and Seeff, 2006) in our series as in other experiences. Such patients may still be candidates for a dual therapy approach as a reasonable therapeutic option, with a sustainable burden of side effects, lower costs and a very high prediction of success, even in the revolutionary era of new antivirals. This could be useful in the current European context, whereby the sustainability of HCV cure is totally in charge of local health services. Interestingly, the latest release of European treatment guidelines stresses the usefulness of extending the lead-in phase with dual therapy to all G1 patients (EASL, 2014; AISF, 2014).

This study has some important limitations: first of all, the sample size, even though it can be assumed as fairly representative of the Italian population of HCV patients (Marcellin *et al.,* 2012); second, the lack of IL28b genotype evaluation for most of patients. These criticisms were mostly due to the real life and non-sponsored design of this institutional study.

In conclusion, in G1 HCV patients, 48 h HCV RNA decay could identify an additional small but significant proportion of patients without RVR who may benefit from dual therapy alone. Evaluation of very early HCV viral kinetics, combined with the conventional 4-week-term evaluation, may well help keep under dual therapy only those patients with a very high chance of success, favoring an early shift to newer options for all the remaining patients.

ACKNOWLEDGEMENTS

We are grateful to the nurses who constantly help us in assisting patients in our wards, and in particular to Mr. Vincenzo Massei. We are also grateful to our colleagues, Dr Tamara Ursini, Dr Alessandro Pieri, Dr Pierluigi Cacciatore, Dr Francesco Di Masi, Dr. Giulio Calella, who helped us in patients enrolment and management.

We are heavily indebted to the Fondazione onlus Camillo de Lellis for Research and Innovation in Medicine and to Ms. Alberta Lolli Ghetti, who gave us logistic support throughout the study period. We are also grateful to the Italian Society of Infectious Diseases (SIMIT) – Abruzzo and Molise Regional Section, for sponsorship.

We also thank our colleagues in the administrative division of Local Health Service in Pescara, who supported and allowed us to design and run this institutional, non-sponsored study.

All the authors declare that they have no conflicts of interest.

REFERENCES

Andriulli A., Di Marco V., Margaglione M., Ippolito A.M., Fattovich G., Smedile A., Valvano M.R., Calvaruso V., Gioffreda D., Milella M., Morisco F., Felder M., Brancaccio G., Fasano M., Gatti P., Tundo P., Barone M., Cozzolongo R., Angelico M., D'Andrea G., Andriulli N., Abate M.L., Mazzella G., Gaeta G.B., Craxi A., Santantonio T. (2014). Identification of naïve HCV-1 patients with chronic hepatitis who may benefit from dual therapy with peg-interferon and ribavirin. *J. Hepatol.* **60**, 16-21.

Asselah T., Marcellin P. (2014). Second-wave IFN-

based triple therapy for HCV genotype 1 infection: simeprevir, faldaprevir and sofosbuvir. *Liver. Int.* (Suppl. 1), 60-68.

Bedossa P., Poynard T. (1996). An algorithm for the grading of activity in chronic hepatitis C. The METAVIR cooperative study group. *Hepatol.* **24**, 289-293.

Beinhardt S., Rutter K., Stättermayer A.F., Ferenci P. (2013). Revisiting the Predictors of a Sustained Virologic Response in the Era of Direct-Acting Antiviral Therapy for Hepatitis C Virus. *Clin. Infect. Dis.* **56**, 118-122.

Bruggmann P., Berg T., Øvrehus A.L., Moreno C., Brandão Mello C.E., Roudot-Thoraval F., Marinho R.T., Sherman M., Ryder S.D., Sperl J., Akarca U., Balik I., Bihl F., Bilodeau M., Blasco A.J., Buti M., Calinas F., Calleja J.L., Cheinquer H., Christensen P.B., Clausen M., Coelho H.S., Cornberg M., Cramp M.E., Dore G.J., Doss W., Duberg A.S., El-Sayed M.H., Ergör G., Esmat G., Estes C., Falconer K., Félix J., Ferraz M.L., Ferreira P.R., Frankova S., García-Samaniego J., Gerstoft J., Giria J.A., Gonçales F.L. Jr, Gower E., Gschwantler M., Guimarães Pessôa M., Hézode C., Hofer H., Husa P., Idilman R., Kåberg M., Kaita K.D., Kautz A., Kaymakoglu S., Krajden M., Krarup H., Laleman W., Lavanchy D., Lázaro P., Marotta P., Mauss S., Mendes Correa M.C., Müllhaupt B., Myers R.P., Negro F., Nemecek V., Örmeci N., Parkes J., Peltekian K.M., Ramji A., Razavi H., Reis N., Roberts S.K., Rosenberg W.M., Sarmento-Castro R., Sarrazin C., Semela D., Shiha G.E., Sievert W., Stärkel P., Stauber R.E., Thompson A.J., Urbanek P., van Thiel I., Van Vlierberghe H., Vandijck D., Vogel W., Waked I., Wedemeyer H., Weis N., Wiegand J., Yosry A., Zekry A., Van Damme P., Aleman S., Hindman S.J. (2014). Historical epidemiology of hepatitis C virus (HCV) in selected countries. *J. Viral. Hepat.* **21** (Suppl. 1), 5-33.

Castera L. (2012). Non invasive method to assess liver disease in patients with hepatitis B and C. *Gastroenterology.* **142**, 1293-1302.

Chopra A., Klein P.L., Drinnan T., Lee S.S. (2013). How to optimize HCV therapy in genotype 1 patients: management of side-effects. *Liver Int.* **33** (Suppl. 1), 30-4.

Durante-Mangoni E., Zampino R., Portella G., Adinolfi L.E., Utili R., Ruggiero G. (2009). Correlates and prognostic value of the first-phase hepatitis C virus RNA kinetics during treatment. *Clin. Infect. Dis.* **49**, 498-506.

European Association for Study of the Liver. (2012). 2011 European Association of the Study of the Liver hepatitis C virus clinical practice guidelines. *Liver Int.* **32** (Suppl. 1), 2-8.

European Association for Study of the Liver. (2014). EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J. Hepatol.* **60**, 392-420.

Hoofnagle J.H., Seeff L.B. (2006). Peginterferon and ribavirin for chronic hepatitis C. *N. Engl. J. Med.* **355**, 2444-2451.

Hoofnagle J.H., Sherker A.H. (2014). Therapy for hepatitis C - the costs of success. *N. Engl. J. Med.* **370**, 1552-1553.

Italian Association for the Study of the Liver (AISF), Coco B., Caraceni P., Aghemo A., Bitetto D., Bruno R., Ciancio A., Marzioni M., Petta S., Rendina M., Valenti L.; Review Board. (2014). Triple therapy with first-generation protease inhibitors for patients with genotype 1 chronic hepatitis C: recommendations of the Italian association for the study of the liver (AISF). *Dig. Liver Dis.* **46**, 18-24.

Mangia A., Bányai T., De Bartolomeo G., Gervain J., Habersetzer F., Mulkay J.P., Ouzan D., Parruti G., Passariello N., Remy A.J., Rizzetto M., Shiffman M.L., Tice A.D., Schmitz M., Tatsch F., Rodriguez-Torres M. (2014). In routine clinical practice, few physicians use early viral kinetics to guide HCV dual therapy treatment decisions. *Liver Int.* **34**, e217-228.

Marcellin P., Cheinquer H., Curescu M., Dusheiko G.M., Ferenci P., Horban A., Jensen D., Lengyel G., Mangia A., Ouzan D., Puoti M., Rodriguez-Torres M., Shiffman M.L., Schmitz M., Tatsch F., Rizzetto M. (2012). High sustained virologic response rates in rapid virologic response patients in the large real-world PROPHESYS cohort confirm results from randomized clinical trials. *Hepatology*. **56**, 2039-2050.

Muir A.J. (2014). The rapid evolution of treatment strategies for hepatitis C. *Am. J. Gastroenterol*. **109**, 628-635.

Parruti G., Polilli E., Sozio F., Cento V., Pieri A., Di Masi F., Mercurio F., Tontodonati M., Mazzotta E., Ceccherini-Silberstein F., Manzoli L., Perno C.F. (2010). Rapid prediction of sustained virological response in patients chronically infected with HCV by evaluation of RNA decay 48h after the start of treatment with pegylated interferon and ribavirin. *Antiviral. Res.* **88**, 124-127.

Poynard T., de Ledinghen V., Zarski J.P., Stanciu C., Munteanu M., Vergniol J., France J., Trifan A., Le Naour G., Vaillant J.C., Ratziu V., Charlotte F.; Fibrosis-TAGS group. (2012). Relative performances of FibroTest, Fibroscan, and biopsy for the assessment of the stage of liver fibrosis in patients with chronic hepatitis C: a step toward the truth in the absence of a gold standard. *J. Hepatol.* **56**, 541-548.

Symonosky V. (1999). The diagnosis of cirrhosis by high resolution ultrasound of the liver surface. *Br. J. Radiol.* **72**, 29-34.