Genotype 4 Hepatitis C Virus Responds Worse than Genotype 1 to 48-Week Combination Treatment with Pegylated Interferon Alpha Plus Ribavirin: a Greek Multi-Centered Study

Savvoula Savvidou[1]*, Dimitrios Chrysagis[2], George V Papatheodoridis[3], Spilios Manolakopoulos[3,4], Christos Triantos[5] and John Goulis[1]

[1]Department of Internal Medicine, Hippocration University Hospital of Thessaloniki, Greece
[2]Department of Internal Medicine, Infectious Diseases Hospital of Thessaloniki, Greece
[3]Department of Internal Medicine, Athens University Medical School, Hippocration General Hospital of Athens, Greece
[4]Department of Gastroenterology, Polyclinic General Hospital of Athens, Greece
[5]Division of Gastroenterology, Department of Internal Medicine, University Hospital of Patras, Greece

Abstract

Background: Prevalence of genotype 4 chronic hepatitis C is increasing in western countries, where response to current combination treatment is still in debate; reports from endemic areas of Middle East show favourable treatment outcomes, while European reports show the contrary. Aim of this retrospective study was to estimate sustained virological response (SVR) of genotype 4 HCV patients in Greece, and to examine possible differences in SVR determinants between genotypes 1 and 4, the two most difficult genotypes to treat.

Methods: Demographic, virological and histological data from 467 consecutive HCV patients from five centers of follow-up were recorded. All patients completed standard combination therapy with pegylated interferon alpha plus weight-based ribavirin, according to current guidelines.

Results: Genotype distribution was: 192(44.8%), 29(6.8%), 130(30.4%), 63(14.7%) and 14(3.3%) for 1, 2, 3, 4 and undefined genotypes, respectively. Baseline characteristics were: 245(57.2%) male, aged 44.8 ± 13.8 years-old, 422(98.6%) white Caucasians, 124(29%) former intravenous drug users, 49(12%) past alcohol abusers, 240(51.5%) overweight and 357(87.7%) naïve. Liver biopsy revealed advanced fibrosis in 58(15.1%) and hepatic steatosis in 133(35.6%) patients. Age (OR 2.1, p=0.007), genotype (OR 3.4, p<0.001), advanced fibrosis (OR 2.9, p=0.003) and naïve status (OR 0.3, p<0.001) were independent prognostic factors for non-response. Comparison between genotype 4 and 1 revealed significant differences in SVR (39.7% vs. 62%, Fisher's exact test, p=0.002). No difference related to any of the demographic, virological or histological variable was able to explain the difference in treatment response.

Conclusion: Genotype 4 chronic hepatitis C in Greece has the worst prognosis in achieving SVR using current combination treatment for 48 weeks. These results challenge the notion, mainly from non-european studies, of a favorable response of genotype 4 compared to genotype 1. Further studies addressing the efficacy of the newer antivirals on the "difficult to treat" genotype 4 should be investigated in the future.

Keywords: Hepatitis C virus; Genotype 4; Treatment outcome; Pegylated interferon alpha; Ribavirin

Abbreviations: HCV: Hepatitis C Virus; Peg-IFNα: Pegylated Interferon-α; SVR: Sustained Virological Response; HIV: Human Immunodeficiency Virus; HBV: Hepatitis B Virus; BMI: Body Mass Index; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; ULN: Upper Limit of Normal; PCR: Polymerase Chain Reaction; HAI: Histology Activity Index; CI: Confidence Intervals; IVDU: Intravenous Drug Use

Introduction

Hepatitis C virus (HCV) is classified into six major genotypes and a series of subtypes, according to its nucleotide sequence [1,2]. Prevalence rates are attributable to the geographical distribution of genotypes, with genotype 4 predominating in Middle East and Africa [1-4]. Recent epidemiological studies report of an increasing prevalence of genotype 4 in western countries, mainly due to imported viral infection from endemic areas [5-7].

Data on the efficacy of current combination therapy with pegylated interferon-α (Peg-IFNα) plus weight-based ribavirin is limited and contradictory; sustained virological response (SVR) rates of more than 60% reported in endemic areas [8-14] are approximately two times higher than those encountered in Europe [15-17]. In Greece, genotype 4 accounts for about 15% of all HCV infections [18-22] and is generally considered as "difficult to treat" in everyday clinical practice.

Aim of this multi-centered study was i) to estimate genotype distribution in Greek consecutive HCV patients, ii) to examine retrospectively the efficacy of standard antiviral therapy, and iii) to compare genotype 1 to genotype 4 response to treatment and examine possible differences in SVR determinants.

Patients and Methods

Patient selection

Databases from five major hepatology units were used to select demographic, virological and histological data from consecutive HCV patients from January 2004 to January 2010. According to inclusion criteria, patients had to be aged above 18 years old, and to have received and completed standard combination therapy by January 2010. Exclusion criteria included concurrent alcohol abuse, co-infection with Human Immunodeficiency (HIV) or Hepatitis B (HBV) virus, and severe comorbid conditions such as chronic kidney failure or thalassemia major. Subcutaneously Peg-IFNα (either Peg-IFNα-2a 180 μg/week or -2b 1.5 μg/kg/week) was administered to all included patients. Ribavirin was co-administered orally on a daily basis at a dose of 800-1200 mg according to patient's baseline characteristics (body weight and genotype) and according to the specific regimen's recommendations. Graph 1 represents a flow diagram demonstrating the selection of patients according to inclusion and exclusion criteria and their stratification according to genotype and treatment algorithm (Graph 1). Patients, who never received nor adhered to more than 80% of the recommended treatment duration, were excluded from the study. Thus, HCV patients who had received treatment totally for less than 20 weeks for genotypes 2 and 3 or less than 40 weeks for genotypes 1 and 4, were not included in final statistical analyses. Furthermore, patients who completed treatment but were lost to follow-up and their SVR had not been determined 24 weeks after the end of treatment, were also excluded from the study.

Demographic data

Databases were used to determine patients' age, gender, origin, probable mode of HCV transmission, history of alcohol consumption, somatometric measurements, and history of previous interferon-based therapy. Past alcohol abuse was defined as a consumption of more than 120g alcohol per week, at least six months prior to the beginning of treatment. Weight and height measurements were used to calculate body mass index (BMI). Patients were defined as overweight if $25 \leq BMI < 30$ kg/m², and obese if $BMI \geq 30$ kg/m². Patients who had never received interferon-based therapy in the past were characterized as naïve.

Laboratory investigations including virology assessment

Baseline serum alanine (ALT) and aspartate aminotransferases (AST) were measured by standard biochemical analyzers. Abnormal values were considered as values just above the upper limit of normal (ULN).

All patients were HCV-RNA positive by qualitative polymerase chain reaction (PCR). HCV RNA was determined by reverse transcriptase-PCR using commercial kits (Amplicor HCV, Roche Diagnostics, Branchburg, NJ) [23]. Baseline high viral load was defined as HCV RNA greater than 800,000 IU/mL.

HCV genotyping was performed with a second-generation reverse hybridization line probe assay (Inno-LiPA HCV II; Belgium), while subtyping was available in only a small proportion of patients.

Liver histology

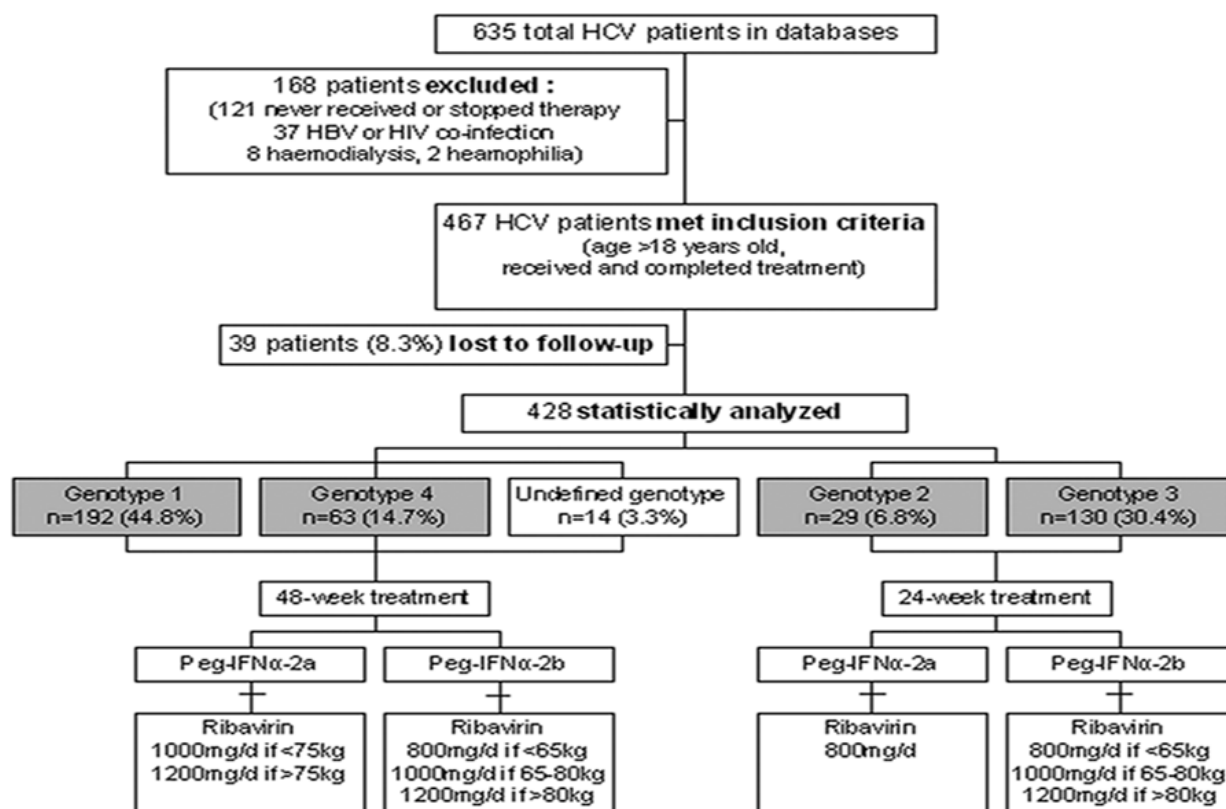Inflammatory activity and fibrosis were assessed according to the

Chart 1: Flow diagram demonstrating patient selection, stratification to different genotypes (in grey) and treatment algorithm.

METAVIR scoring system (4 stages for activity: A0-A3 and 5 stages for fibrosis: F0-F4) or the modified Ishak score (Histology Activity Index (HAI) scale 0-18 and fibrosis scale 0-6) [24,25]. Severe inflammation was considered as having either A3 or HAI>12. Advanced fibrosis was defined as METAVIR stage $\geq$ F3 or HAI fibrosis scale $\geq$ 4, while cirrhosis was defined as having METAVIR stage F4 or HAI stages $\geq$ 5.

Steatosis was semi-quantified by determining the proportion of hepatocytes containing fat droplets. According to Brunt's classification [26], specimens were assigned a grade (0 to III) based upon the percentage of affected hepatocytes. Grade 0 was considered as absence of hepatic steatosis, while grades I to III was considered as presence of hepatic steatosis.

Treatment outcome

Primary endpoint of the study was SVR, defined as undetectable HCV-RNA 24 weeks after the end of treatment. Relapsers (undetectable HCV-RNA in the end of treatment but positive after 24 weeks) and non-responders (positive HCV-RNA by the end of treatment) were both considered as patients who failed to achieve SVR and, thus, as treatment failures. Rapid virological and early virological responses with detection of HCV-RNA at weeks 4 and 12, respectively, had not been assessed.

Severe adverse effects including mainly anemia and neutropenia were recorded and further handled by dose reductions or supplementation of erythropoietin. Patients, who constantly, throughout the recommended treatment duration, received more than 80% of the initial dose for both regimens, were recorded as adherent to standard dosage.

Statistical analysis

Statistical tests of χ^2, Fisher's exact test, t-Student test and one-way ANOVA were used for group comparisons, as appropriate. Chi-square and Fisher's exact test were used for categorical variables, t-Student for normally distributed continuous variables of two independent samples, while one-way ANOVA was performed for normally distributed continuous variables of more than two independent samples. Treatment outcome was analyzed as the depended dichotomous categorical variable and, thus, patients were grouped into those achieving SVR and those who failed to achieve SVR (both relapsers and non-responders). Univariate and multivariate logistic regression analyses were performed in order to determine the Odds Ratios of various independent factors for treatment failure, as well as their 95% Confidence Intervals (CI). Finally, subgroup analysis included only genotype 1 and 4 HCV patients for further statistical comparison. All statistical analyses were made using SPSS v11.5. P values were considered statistical significant at the 0.05 level.

Results

Baseline patient, viral and histological characteristics

The study's flow diagram is demonstrated in Chart 1. From the original study sample of 635 consecutive HCV patients recorded in databases, 467 patients met inclusion criteria. Chart 1 also demonstrates the distribution of genotypes, which was found not to have any statistically significant difference from the original study population (χ^2, df=4, p=0.902). Analyzing the characteristics of patients excluded, it was found that genotype 1 and 4 HCV patients and patients with advanced fibrosis were more prone to drop out, probably due to longer duration of treatment and non-compliance, respectively.

A total of 428 patients were finally included in statistical analyses,

as 39 patients (8.3%) were lost to follow-up and SVR could not be determined. Table 1 summarizes baseline patient characteristics in total and according to genotype. All patients were white Caucasians who lived and worked in Greece, except from six patients (1.4%) with Egyptian origin. Approximately, one third of patients had acquired HCV by intravenous drug use and above 50% of HCV patients were overweight. The majority of patients had abnormal aminotransferases at the beginning of therapy (ALT>ULN 92.2% and AST>ULN 82.9%). Liver histology was available in 383 (89.5%) HCV patients, with 58 (15.1%) patients having evidence of advanced fibrosis, and 18 (4.7%) severe necro-inflammatory activity. Hepatic steatosis was distributed to 241 (64.4%), 84 (22.5%), 39 (10.4%) and 10 (2.7%) for grades 0, I, II and III, respectively.

Genotype distribution and comparison of different genotypes

Genotype distribution was: 192 (44.8%), 29 (6.8%), 130 (30.4%), 63 (14.7%) and 14 (3.3%) for 1, 2, 3, 4 and undefined genotypes, respectively. Genotype distribution is shown in both Table 1 and Graphs 1 and 2. Genotype 4 was on the third place with a prevalence of 14.7% (n=63 patients), while data on subtyping was available for only 13 (20.6%) of genotype 4 HCV patients (Graph 2). There were 5 different subtypes found in the cohort of genotype 4 HCV patients, namely a, b, c/d, e and h. Subtype 4c/d was the most prevalent (n=6), but was found not to be associated with treatment outcome.

Statistical comparison between different genotypes revealed no significant differences in gender (χ^2, df=4, p=0.974), history of past alcohol consumption (χ^2, df=4, p=0.078), BMI (one-way ANOVA, df=4, p=0.423), baseline viral load (χ^2, df=4, p=0.421), naïve status (χ^2, df=4, p=0.189) or histological features concerning fibrosis, necro-inflammatory activity and steatosis (Table 1). On the other hand, the observed statistically significant differences in age and mode of transmission were attributable to the fact that genotype 3 HCV patients were significantly younger and mostly former IVDUs. Furthermore, abnormal baseline ALT was seen more often in genotypes 1 and 3, while Peg-IFNα-2b had been preferred mostly for the longer treatments of genotype 1 and 4 patients.

SVR rates were 62.0%, 75.9%, 83.9% and 39.7% for genotypes 1, 2, 3 and 4, respectively. SVR was significantly lower for genotypes 1 and 4 compared to genotypes 2 and 3 (χ^2, df=4, p<0.001).

Determinants of treatment outcome

Factors associated with treatment outcome were analyzed statistically with univariate and multivariate logistic regression analysis (Table 2). In univariate analysis, history of IVDU and naïve status were positively associated with SVR, while age older than 40 years, genotypes 1 or 4, advanced fibrosis and presence of hepatic steatosis were found to be associated with treatment failure.

Statistical analysis with multivariate logistic regression revealed that age above 40 years old (Odds Ratio=2.1, p=0.007, 95% CI:1.2-3.7), genotype 1 or 4 (Odds Ratio=3.4, p<0.001, 95% CI:1.9-6.1), advanced fibrosis (Odds Ratio=2.9, p=0.003, 95% CI:1.5-5.9) and naïve status (Odds Ratio=0.3, p<0.001, 95% CI:0.1-0.5) were independent factors for non-response to standard combination treatment (Hosmer-Lemeshow test 0.985>0.05). Naïve status was the only variable that was positively associated with SVR, while age, genotypes 1 or 4, and advanced fibrosis was negative factors for SVR.

### Genotype 4 to genotype 1 comparison

Further statistical subgroup analysis included only genotype 4

	Total (n=428)	Genotype 1 (n=192)	Genotype 2 (n=29)	Genotype 3 (n=130)	Genotype 4 (n=63)	Undefined (n=14)	p values*	p values**
Demographics								
Male gender (%)	245 (57.2)	108 (56.3)	16 (55.2)	76 (58.5)	36 (57.1)	9 (64.3)	0.974	0.901
Age (years)	44.8 ± 13.8	47.1 ± 13.1	53.3 ± 11.6	38.6 ± 13.1	47.1 ± 13.1	42.8 ± 16.2	<0.001	0.975
Age >40 years old (%)	241 (57.4)	126 (66.3)	23 (79.3)	47 (36.7)	41 (66.1)	4 (36.4)	<0.001	0.978
Mode of transmission							<0.001	0.092
IVDU	124 (29.0)	44 (22.9)	4 (13.8)	62 (47.7)	10 (15.9)	4 (28.6)		
Transfusion	81 (18.9)	36 (18.8)	8 (27.6)	18 (13.8)	17 (27.0)	2 (14.3)		
Multiple sex partners	15 (3.5)	11 (5.7)	0 (0)	4 (3.1)	0 (0)	0 (0)		
Unknown	208 (48.6)	101 (52.6)	17 (58.6)	46 (35.4)	36 (57.1)	8 (57.1)		
Past alcohol abuse (%)	49 (12.0)	18 (9.5)	1 (3.4)	17 (13.8)	11 (18.3)	2 (33.3)	0.078	0.062
Somatometrics								
BMI (kg/m^2)	23.30 ± 3.80	23.36 ± 3.92	25.29 ± 4.33	22.88 ± 3.59	23.03 ± 3.66	24.50 ± 2.12	0.423	0.695
BMI (categorical)							0.088	0.522
Normal BMI	144 (38.5)	62 (34.8)	9 (32.1)	49 (43.4)	23 (43.4)	1 (50.0)		
Overweight	189 (50.5)	90 (50.6)	14 (50.0)	61 (54.0)	23 (43.4)	1 (50.0)		
Obese	41 (11)	26 (14.6)	5 (17.9)	3 (2.7)	7 (13.2)	0 (0)		
Virology								
High viral load (%)	92 (44.9)	34 (37.8)	6 (42.9)	36 (52.9)	14 (48.3)	2 (50.0)	0.431	0.316
ALT >ULN (%)	378 (92.2)	173 (95.1)	24 (82.8)	122 (94.6)	51 (83.6)	8 (88.9)	0.011	0.064
AST >ULN (%)	340 (82.9)	153 (84.1)	22 (75.9)	107 (82.9)	50 (82.0)	8 (88.9)	0.835	0.702
Histology								
Severe inflammation (%)	18 (4.7)	11 (6.0)	0 (0)	3 (2.7)	2 (3.9)	2 (18.2)	0.118	0.570
Advanced fibrosis (%)	58 (15.1)	27 (14.5)	4 (16.0)	16 (14.5)	10 (19.6)	1 (9.1)	0.877	0.375
Hepatic steatosis	133 (35.6)	60 (33.5)	16 (36.0)	37 (33.9)	21 (41.2)	6 (60)	0.439	0.313
Treatment								
Naïve (%)	357 (87.7)	159 (84.6)	26 (89.7)	116 (92.8)	50 (84.7)	6 (100)	0.189	0.975
Type of PegIFNα							<0.001	0.712
-2a	123 (30.3)	44 (23.2)	15 (51.7)	52 (40.9)	11 (20.8)	1 (14.3)		
-2b	283 (69.7)	146 (76.8)	14 (48.3)	75 (59.1)	42 (79.2)	6 (85.7)		
Adherence to dose (%)	373 (87.1)	160 (83.3)	25 (86.2)	120 (92.3)	55 (87.3)	13 (92.9)	0.198	0.472
SVR (%)	283 (66.1)	119 (62.0)	22 (75.9)	109 (83.8)	25 (39.7)	8 (57.1)	<0.001	0.002

* for comparisons between 1,2,3,4 and undefined genotypes
** for comparisons between genotypes 1 and 4

Table 1: Baseline patient characteristics according to genotype and comparison of genotype 1 to genotype 4 (grey columns).

Chart 2: Genotype distribution (pie chart) and subtypes of genotype 4 HCV patients.
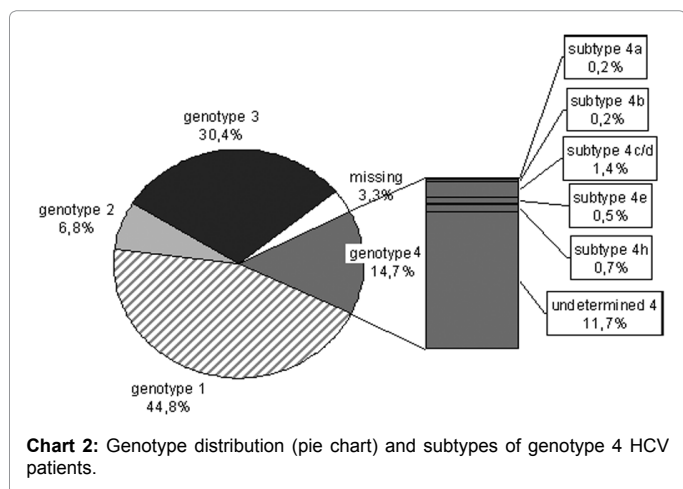
(n=63) and genotype 1 patients (n=192). SVR was lower for genotype 4 (39.7% vs. 62.0%, Fisher's exact test, p=0.002), although no other statistical significant differences in SVR determinants were recorded (Table 1).

In the multivariate logistic regression subgroup analysis, which included only genotype 4 and genotype 1 HCV patients, treatment failure was independently associated with genotype 4 (OR 4.28, 95%CI 2.03-8.99, p<0.001), older age than 40 years (OR 2.28, 95%CI 1.18-4.44, p=0.015), advanced fibrosis (OR 2.57, 95%CI 1.06-6.26, p=0.037) and previous interferon-based therapy (OR 5.77, 95%CI 2.27-14.71, p<0.001). Hosmer-Lemeshow goodness-of-fit test for this multivariate logistic regression model was 0.817 (>0.05).

Discussion

Recent epidemiological studies report that prevalence of genotype 4 chronic hepatitis C is increasing in western countries, and two major causes have been identified so far; first, a substantial population from endemic areas of Middle East immigrate across Europe every year and, secondly, genotype 4 has been found to widespread among IVDUs in several European countries [1,6,7]. In Greece, prevalence of genotype 4 is amongst the highest reported within Europe, estimated around 15% [18-22]. Similarly, a percentage of 14.7% was confirmed in our study cohort of consecutive HCV patients.

Data for the efficacy of standard combination therapy with Peg-IFNα plus ribavirin in genotype 4 comes mainly from endemic areas

Variable	Univariate analysis			Multivariate analysis		
	Odds Ratio	95% CI	p values	Odds Ratio	95% CI	p values
Male gender	0.918	0.613-1.375	0.679			
Age ≥40 years	3.405	2.168-5.350	<0.001	2.127	1.233-3.670	0.007
IVDU	0.317	0.190-0.531	<0.001			
Past alcohol abuse	1.277	0.690-2.363	0.436			
BMI >25 kg/m^2	1.329	0.859-2.055	0.201			
Genotype 1 or 4	3.606	2.238-5.812	<0.001	3.395	1.900-6.064	<0.001
High viral load	1.180	0.675-2.061	0.562			
Severe inflammation	2.025	0.784-5.233	0.146			
Advanced fibrosis	4.021	2.247-7.197	<0.001	2.926	1.457-5.875	0.003
Hepatic steatosis	2.884	1.842-4.514	<0.001			
Naïve status	0.149	0.077-0.289	<0.001	0.251	0.199-0.528	<0.001
PegIFNα-2a vs.-2b	0.939	0.598-1.473	0.783			

Table 2: Factors associated with treatment failure in the total cohort of HCV patients.

such as Egypt [8,9,12,14], Saudi Arabia [10], Kuwait [13] and Quatar [11]. The reported SVRs range between 59.7 and 67.8%. On the contrary, similar European studies report significantly lower SVRs, without being able to explain these observations by differences in patient baseline characteristics like age, naïve status or fibrosis stage [15-17]. In our study, genotype 4 HCV patients achieved SVR in 39.7%, similar to previously reported percentages in Greece [20,21]. This also confirms our primary observation that genotype 4 in Greece is considered as "difficult to treat" in everyday clinical practice.

Studies looking at predictive factors on genotype 4 treatment outcome are relatively scarce [1]. Besides presence of advanced fibrosis, treatment outcome in genotype 4 has been associated with insulin resistance, hepatic steatosis and adiponectin changes, as in other genotypes [27-32]. Even though neither insulin resistance nor adiponectin levels were addressed in our study, indirect evidence, estimated by histologic presence of hepatic steatosis and measurement of BMI, showed that these factors were probably not related to genotype's 4 worse prognosis.

Recently, the identification of interleukin 28B (IL28B) polymorphism has proved to be a significant determinant of HCV response to interferon-based therapies [1,33]. Even though IL28B has been found to be associated with SVR in some reports concerning genotype 4 as well [33-35], it still remains unclear whether the difference in SVR observed between endemic and European studies is related to ethnicity, HCV subtype, the mode of transmission or the IL-28B genotype [1,36]. Furthermore, distribution of IL28B polymorphisms varies between different populations worldwide and could help explain the heterogeneity in response to treatment in different ethnic or racial groups, but this still needs to be confirmed in large epidemiological studies in the Middle East and Europe. In our study, the observed worse response of genotype 4 when compared to genotype 1 HCV patients could theoretically and partly been explained by differences in IL28B polymorphism. However, this identification could not have been performed retrospectively.

In conclusion, having in mind that major limitation of all retrospective studies is that they can easily be subjected to selection bias, and trying to interpret our observations with caution, the results of our study confirm that prevalence of genotype 4 has increased to 15% in southern Europe, and indicate that genotype 4 has a non-favorable response to standard combination treatment with Peg-IFNα plus ribavirin. This may be attributable to IL28B polymorphisms, but further studies have to address this hypothesis. The role of newer drugs like protease inhibitors and direct acting antivirals seem promising in genotype 4 as well [37,38] and should also be investigated in the future.

References

1. Khattab MA, Ferenci P, Hadziyannis SJ, Colombo M, Manns MP, et al. (2011) Management of hepatitis C virus genotype 4: recommendations of an international expert panel. J Hepatol 54: 1250-1262.

2. Antaki N, Craxi A, Kamal S, Moucari R, Van der Merwe S, et al. (2010) The neglected hepatitis C virus genotypes 4, 5 and 6: an international consensus report. Liver Int 30: 342-355.

3. Kamal SM (2009) Hepatitis C genotype 4 therapy: increasing options and improving outcomes. Liver Int 29 Suppl 1: 39-48.

4. Nguyen MH, Keeffe EB (2005) Prevalence and treatment of hepatitis C virus genotypes 4, 5, and 6. Clin Gastroenterol Hepatol 3: S97-97S101.

5. Eriksen MB, Jørgensen LB, Krarup H, Laursen AL, Christensen PB, et al. (2010) Molecular and epidemiological profiles of hepatitis C virus genotype 4 in Denmark. J Med Virol 82: 1869-1877.

6. de Bruijne J, Schinkel J, Prins M, Koekkoek SM, Aronson SJ, et al. (2009) Emergence of hepatitis C virus genotype 4: phylogenetic analysis reveals three distinct epidemiological profiles. J Clin Microbiol 47: 3832-3838.

7. Sereno S, Perinelli P, Laghi V (2009) Changes in the prevalence of hepatitis C virus genotype among Italian injection drug users-relation to period of injection started. J Clin Virol 45: 354-357.

8. Kamal SM, Ahmed A, Mahmoud S, Nabegh L, El Gohary I, et al. (2011) Enhanced efficacy of pegylated interferon alpha-2a over pegylated interferon and ribavirin in chronic hepatitis C genotype 4A randomized trial and quality of life analysis. Liver Int 31: 401-411.

9. El Makhzangy H, Esmat G, Said M, Elraziky M, Shouman S, et al. (2009) Response to pegylated interferon alfa-2a and ribavirin in chronic hepatitis C genotype 4. J Med Virol 81: 1576-1583.

10. Dahlan Y, Ather HM, Al-ahmadi M, Batwa F, Al-hamoudi W (2009) Sustained virological response in a predominantly hepatitis C virus genotype 4 infected population. World J Gastroenterol 15: 4429-4433.

11. Derbala MF, El Dweik NZ, Al Kaabi SR, Al-Marri AD, Pasic F, et al. (2008) Viral kinetic of HCV genotype-4 during pegylated interferon alpha 2a: ribavirin therapy. J Viral Hepat 15: 591-599.

12. Khattab M, Eslam M, Sharwae MA, Shatat M, Ali A, et al. (2010) Insulin resistance predicts rapid virologic response to peginterferon/ribavirin combination therapy in hepatitis C genotype 4 patients. Am J Gastroenterol 105: 1970-1977.

13. Varghese R, Al-Khaldi J, Asker H, Fadili AA, Al Ali J, et al. (2009) Treatment of chronic hepatitis C genotype 4 with peginterferon alpha-2a plus ribavirin. Hepatogastroenterology 56: 218-222.

14. Khuroo MS, Khuroo MS, Dahab ST (2004) Meta-analysis: a randomized trial of peginterferon plus ribavirin for the initial treatment of chronic hepatitis C genotype 4. Aliment Pharmacol Ther 20: 931-938.

15. López-Alonso G, Agreda M, Devesa MJ, Cuenca F, Suárez A, et al. (2008) [Results of the treatment of chronic hepatitis C genotype 4--a comparative analysis with genotype 1]. Rev Esp Enferm Dig 100: 208-211.

16. Roulot D, Bourcier V, Grando V, Deny P, Baazia Y, et al. (2007) Epidemiological characteristics and response to peginterferon plus ribavirin treatment of hepatitis C virus genotype 4 infection. J Viral Hepat 14: 460-467.

17. de Galocsy C, Kaufman L, Tomasovic S, Delwaide J, Nevens F (2010) Hepatitis C genotype 4 response rate to pegylated interferon and ribavirin treatment in Belgium is similar to genotype 1. Acta Gastroenterol Belg 73: 229-234.

18. Katsoulidou A, Sypsa V, Tassopoulos NC, Boletis J, Karafoulidou A, et al. (2006) Molecular epidemiology of hepatitis C virus (HCV) in Greece: temporal trends in HCV genotype-specific incidence and molecular characterization of genotype 4 isolates. J Viral Hepat 13: 19-27.

19. Savvas SP, Koskinas J, Sinani C, Hadziyannis A, Spanou F, et al. (2005) Changes in epidemiological patterns of HCV infection and their impact on liver disease over the last 20 years in Greece. J Viral Hepat 12: 551-557.

20. Elefsiniotis IS, Pavlidis C, Dimitroulopoulos D, Vezali E, Mihas C, et al. (2009) Differential viral kinetics in treated genotype 4 chronic hepatitis C patients according to ethnicity. J Viral Hepat 16: 738-742.

21. Dimitroulopoulos D, Elefsiniotis I, Pavlidis C, Xinopoulos D, Tsamakidis K, et al. (2010) European vs. Egyptian HCV-4 patients with elevated baseline HCV RNA, treated with PEG-IFN-α2a and ribavirin: the role of rapid and early virologic response. Hepat Mon 10: 193-198.

22. Papastergiou V, Dimitroulopoulos D, Skorda L, Lisgos P, Ketikoglou I, et al. (2012) Predictors of sustained virological response in Greek and Egyptian patients with hepatitis C genotype 4: does ethnicity matter? J Med Virol 84: 1217-1223.

23. Elkady A, Tanaka Y, Kurbanov F, Sugauchi F, Sugiyama M, et al. (2010) Performance of two Real-Time RT-PCR assays for quantitation of hepatitis C virus RNA: evaluation on HCV genotypes 1-4. J Med Virol 82: 1878-1888.

24. Bedossa P, Poynard T (1996) An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. Hepatology 24: 289-293.

25. Brunt EM (2000) Grading and staging the histopathological lesions of chronic hepatitis: the Knodell histology activity index and beyond. Hepatology 31: 241-246.

26. Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR (1999) Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 94: 2467-2474.

27. Moucari R, Ripault MP, Martinot-Peignoux M, Voitot H, Cardoso AC, et al. (2009) Insulin resistance and geographical origin: major predictors of liver fibrosis and response to peginterferon and ribavirin in HCV-4. Gut 58: 1662-1669.

28. Legrand-Abravanel F, Colson P, Leguillou-Guillemette H, Alric L, Ravaux I, et al. (2009) Influence of the HCV subtype on the virological response to pegylated interferon and ribavirin therapy. J Med Virol 81: 2029-2035.

29. Moucari R, Asselah T, Cazals-Hatem D, Voitot H, Boyer N, et al. (2008) Insulin resistance in chronic hepatitis C: association with genotypes 1 and 4, serum HCV RNA level, and liver fibrosis. Gastroenterology 134: 416-423.

30. Khattab MA, Abdel-fattah ME, Eslam M, Abdelaleem A, Abdelaleem RA, et al. (2010) Hepatic steatosis in genotype 4 chronic hepatitis C patients: implication for therapy. J Clin Gastroenterol 44: 707-712.

31. Gad RR, Males S, El Makhzangy H, Shouman S, Hasan A, et al. (2008) Predictors of a sustained virological response in patients with genotype 4 chronic hepatitis C. Liver Int 28: 1112-1119.

32. Derbala M, Rizk N, Al-Kaabi S, Amer A, Shebl F, et al. (2009) Adiponectin changes in HCV-Genotype 4: relation to liver histology and response to treatment. J Viral Hepat 16: 689-696.

33. Asselah T, De Muynck S, Broët P, Masliah-Planchon J, Blanluet M, et al. (2012) IL28B polymorphism is associated with treatment response in patients with genotype 4 chronic hepatitis C. J Hepatol 56: 527-532.

34. Abdo AA, Al-Ahdal MN, Khalid SS, Helmy A, Sanai FM, et al. (2013) IL28B polymorphisms predict the virological response to standard therapy in patients with chronic hepatitis C virus genotype 4 infection. Hepatol Int 7: 533-538.

35. De Nicola S, Aghemo A, Rumi MG, Galmozzi E, Valenti L, et al. (2012) Interleukin 28B polymorphism predicts pegylated interferon plus ribavirin treatment outcome in chronic hepatitis C genotype 4. Hepatology 55: 336-342.

36. Rallón NI, Naggie S, Benito JM, Medrano J, Restrepo C, et al. (2010) Association of a single nucleotide polymorphism near the interleukin-28B gene with response to hepatitis C therapy in HIV/hepatitis C virus-coinfected patients. AIDS 24: F23-29.

37. Benhamou Y, Moussalli J, Ratziu V, Lebray P, De Backer K, et al. (2013) Telaprevir activity in treatment-naive patients infected hepatitis C virus genotype 4: a randomized trial. J Infect Dis 208: 1000-1007.

38. Esmat G, El Raziky M, El Kassas M, Hassany M, Gamil ME (2012) The future for the treatment of genotype 4 chronic hepatitis C. Liver Int 32: 146-150.